SYNTHESIS OF HETEROCYCLIC NITROXIDES WITH AN IMPROVED YIELD BY INVESTIGATING THE TETRAALKYLATION OF *N*-BENZYLPHTHALIMIDE.

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- Jayawardena, V.C.; Fairfull-Smith, K.E.; Bottle, S.E. "Improving the synthesis of isoindoline nitroxides by step-wise Grignard addition"

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Papers

- Jayawardena, V.C.; Fairfull-Smith, K.E.; Bottle, S.E. *Aust. J. Chem.* **2013**, *66*, 619-625.
  (Full paper is attached in the Appendices)
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- Jayawardena, V.C.; Fairfull-Smith, K.E.; Bottle, S.E. *Synthesis of Pyridine-Annulated Heterocyclic Nitroxides with an Improved Yield* (Manuscript in preparation).

Abstract

The progress of the Grignard tetraethylation of *N*-benzylphthalimide, the crucial step in the synthesis of 1,1,3,3-tetraethylisoindolin-2-yloxyl (TEIO), was found to be limited by the formation of hitherto unreported mono- and diethyl side products which do not lead to the desired tetraethyl adduct (2-benzyl-1,1,3,3-tetramethylisoindoline). Although the ethyliminium ion is considered as the precursor intermediate for the formation of the tetraethyl adduct, the competitive conversion of the iminium ion into the side products that do not lead to the desired target is the likely cause for the limited yield. The overall synthetic yield of the tetraethyl adduct was improved to 49% over two steps by employing a step-wise addition sequence starting from *N*-benzylphthalimide. The two step synthesis offers a practical preparative scale alternative to the current approach. Grignard tetramethylation of *N*-benzylphthalimide was also investigated in a similar manner to the methodology used to investigate tetraethylation and it was found that yield of tetramethyl adduct was also limited due to the formation of side products. It was shown that one of these side products (an exocyclic enamide) is the precursor for the formation of a purple coloured intractable material, which was identified as a major byproduct of the Grignard methylation of *N*-benzylphthalimide. Various attempts to improve the yield of the tetramethyl adduct were unsuccessful, either by a step-wise addition method or by modifying the experimental conditions, due to the unavoidable formation of the purple coloured oligomeric mixture.

The synthesis of novel, unsubstituted, pyridine-annulated pyrrolidine nitroxides was carried out (starting from *N*-benzylcinchomeronic imide) via a Grignard approach by exploiting the knowledge gathered during the alkylation of isoindoline systems.

Synthesis of the tetraethyl-pyridine adduct was not achievable due to the formation of numerous side products such as 1,1-diethyl adducts and some ring substituted products, which are unlikely to be intermediates on the pathway to form the expected tetraethyl adduct. The synthesis of the tetramethyl-pyridine adduct was however achieved successfully with an isolated yield of 18%. Hydrogenation of the tetramethyl-pyridine adduct followed by oxidation gave two novel pyridine annulated heterocycic nitroxides with overall yields of ~15%. This overall yield is much improved compared to the existing synthesis of substituted pyridine-annulated pyrrolidine nitroxides via electrocyclic reactions.

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List of Abbreviations

A-T	ataxia telangiectasia
CTMIO	5-Carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl
DCM	dichloromethane
DCTMIO	5,6-dicarboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
EPR	electron paramagnetic resonance
Et	ethyl
EtMgI	ethylmagnesium iodide
HMBC	heteronuclear-multiple-bond correlation
HPLC	high pressure liquid chromatography
IC	internal conversion
ISC	intersystem crossing
IR	infrared
LUMO	lowest unoccupied molecular orbital
MALDI	matrix-assisted laser desorption ionization
*m*CPBA	*meta*-chloroperoxybenzoic acid
Me	methyl
MeLi	methyllithium
MeMgI	methylmagnesium iodide
MPLC	medium pressure liquid

	chromatography
MRI	magnetic resonance imaging
NMP	nitroxide-mediated polymerization
NMR	nuclear magnetic resonance
NOESY	nuclear overhauser effect spectroscopy
OMe	methoxy
RAFTP	reversible addition fragmentation chain transfer polymerization
rt	room temperature
SDSL	site-directed spin labelling
SEC	size exclusion chromatography
TEIO	1,1,3,3-tetraethylisoindolin-2-yloxyl
TEMPO	2,2,6,6-tetramethylpiperidinyloxyl
THF	tetrahydrofuran
TLC	thin layer chromatography
TMAO	1,1,3,3-tetramethyl-2,3-dihydro-azaphenalen-2-yloxyl
TMDBIO	1,1,3,3-tetramethyldibenzoisoindolin-2-yloxyl
TMIO	1,1,3,3-tetramethylisoindolin-2-yloxyl
UV	ultraviolet
VR	vibrational relaxation

Statement of Original Authorship

The work contained in this thesis has not been previously submitted to meet requirements for an award at this or any other higher education institution. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made.

Signature: QUT Verified Signature

Date: 05/11/2014

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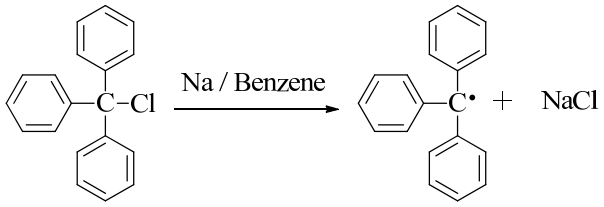
Chapter 1: Introduction

1.1 FREE RADICALS

Free radical containing compounds are defined as neutral molecules containing one or more unpaired electrons.[1] The presence of an unpaired electron imparts a property called paramagnetism, whereby the unpaired electrons are weakly attracted by a magnetic field. In the period from 1840 to 1865, many literature procedures reported the preparation of methyl and ethyl radicals.[2] However, it was later recognized that all these reported literature procedures had given dimers, such as ethane from two methyl radicals and butane from two ethyl radicals.[2] In 1900, Moses Gomberg demonstrated that radicals can be produced by treating a halide with a metal and this procedure remains as one of the standard procedures for the preparation of stable free radicals to date.[2]



1.1 **1.2**

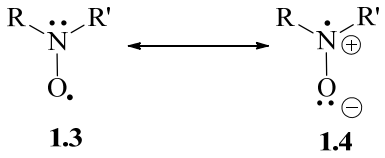
Scheme 1.1 Gomberg's method of synthesizing radicals.[2]

Free radicals play an important role in several fields like combustion, biochemistry, polymerization, atmospheric chemistry, plasma chemistry, human physiology, etc.,[3] and are usually considered to be highly reactive intermediates with short lifetimes. This is true for most free radicals, but there are some free radicals which are long-lived and isolable in pure form by traditional chemical methods.[4] Probably the most important example of this type is the nitroxide radicals, which are derivatives of

nitrogen oxide and which contain a disubstituted nitrogen atom bound to a one-valent oxygen atom.

1.2 NITROXIDE RADICALS

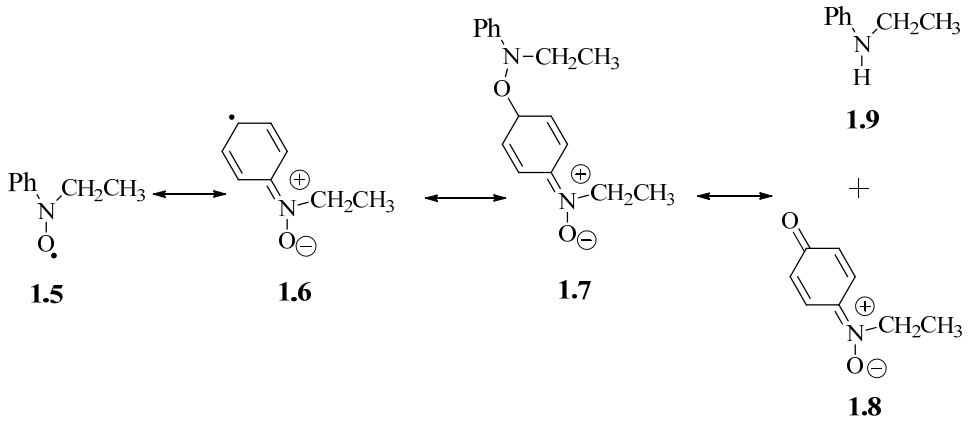
Nitroxides, also commonly known as nitroxyl, or less frequently aminoxyl radicals, are stable free radicals. Nitroxides show resistance towards dimerisation due to the high electronegativity of N and O atoms and the high delocalization energy possessed by the unpaired electron of the oxygen. This means the radical centre of the nitroxide is thermodynamically more stable than the O-O bond that would be formed in the dimerised product.[4]



1.3 **1.4**

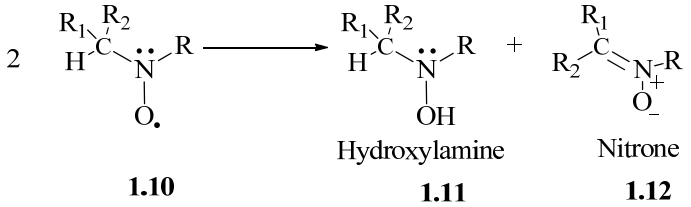
Scheme 1.2 Resonance stabilization of a nitroxide radical.

As well as thermodynamic factors, kinetic factors are also responsible for the overall stability and persistence of the nitroxide radical. Of particular importance are the substituents directly attached to the nitrogen of the nitroxide, as these control its kinetic stability. For example, when a phenyl group is attached to the nitrogen atom of the nitroxide, the free radical life time is shortened, as bimolecular combination produces a diamagnetic (non-radical) product. This occurs readily even though the delocalization of the radical onto the aromatic ring improves the thermodynamic stability of the nitroxide (Scheme 1.3).



Scheme 1.3 Bimolecular degradation of a nitroxide.[4]

Bimolecular reactions of nitroxides can also occur when the *N*-substituent carries a C-H bond. In this case, disproportionation takes place to form a hydroxylamine and a nitrone (Scheme 1.4).[4]



Scheme 1.4 Disproportionation of a nitroxide.[4]

The most stable form of nitroxides contains substituents which lack α-hydrogen atoms, thus preventing disproportionation reactions. In addition, these substituents provide steric hindrance around the nitroxide moiety, so the nitroxide is kinetically stabilized.[4] Examples for such nitroxides are TEIO (1,1,3,3-tetraethylisoindoline-2-yloxyl, **1.13**), TEMPO (2,2,6,6-tetramethylpiperidin-1-yloxyl, **1.14**) and TMIO (1,1,3,3-tetramethylisoindoline-2-yloxyl, **1.15**) etc. (Figure 1.1)

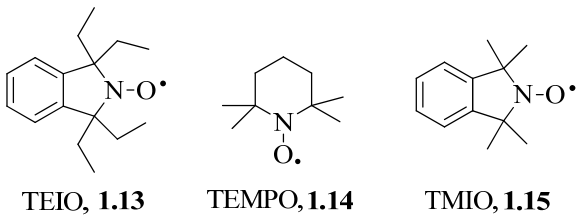


Figure 1.1 Structures of stable nitroxides

Nitroxides have become a significant area of research over the past 40 years owing to their remarkable physical and chemical properties.

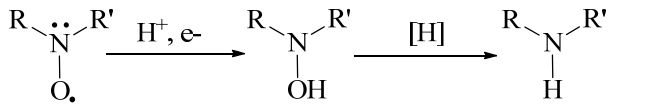
1.3 CHEMICAL PROPERTIES OF NITROXIDES

Nitroxides mainly undergo 3 types of reactions; oxidation, reduction and radical trapping.

1.3.1 Reduction

1.3.1.1 Chemical Reduction

Nitroxides can undergo a one electron reduction to give hydroxylamines in the presence of mild reducing agents or a complete two electron reduction to a secondary amine in the presence of strong reducing agents. Each of these processes require both an electron donor and a H^+ donor, thus these reductions will take place in an acidic organic medium or in water.[5] The reduction of a nitroxide (**1.3**, Scheme 1.5) to its corresponding hydroxylamine (**1.16**, Scheme 1.5) is a reversible process and the reverse reaction can occur either under mild oxidizing conditions (such as atmospheric oxygen)[5] as well as under stronger oxidizing conditions (such as silver oxide, ferricyanide, H_2O_2, permanganate etc.).[6]



Nitroxide **1.3** — Hydroxylamine **1.16** — Secondary amine **1.17**

Scheme 1.5 Reduction of a nitroxide

Complete reduction to the secondary amine occurs under strong reducing agents such as hydrogenation with Raney nickel, heating acidic solutions of nitroxides in the presence of zinc chloride, etc.[4] Conversion of the secondary amine to the nitroxide can be achieved using stronger oxidizing conditions compared to those required for

oxidation of the hydroxylamine. A typical procedure would involve the reaction of an appropriate secondary amine with H_2O_2 in the presence of a sodium tungstate catalyst.[4]

1.3.1.2 Biological Reduction

In biological systems, nitroxides usually undergo reversible reactions to give hydroxylamines. This reduction is frequently considered the principal metabolic pathway for nitroxides *in vivo*. However, there are several factors that affect the rate of reduction of nitroxides in biological systems.

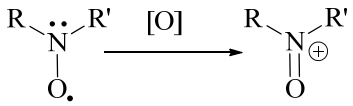
The structure of the nitroxide has a clear influence on the rate of its reduction in biological systems. For example, 6-membered nitroxides undergo reduction much faster than the 5-membered nitroxides.[7,8] This is mainly due to the greater conformational flexibility of the 6-membered rings, which allows the reducing agents to approach the reaction site much more efficiently. Functional groups attached to the nitroxide are also a major factor which controls the bioreduction of nitroxides. When carboxylic acid groups are attached to the nitroxides, they have shown a resistance towards bioreduction by ascorbate in rat hepatocytes.[8] The reason for this resistance is that at biological pH, carboxylic acid groups usually appear in carboxylate form, which hinders the addition of another negative charge (an electron) to the nitroxide.

Apart from the physical and chemical characteristics of nitroxides, some other factors like cellular redox environment, the type of cell and cell permeability also have an impact on the rate of hydroxylamine formation *in vivo*.[5,9] Of these, oxygen concentration of the cellular redox environment plays a major factor in the rate of bioreduction. Higher cellular oxygen concentration decreases the rate of the formation of hydroxylamines,[10] as the reverse reaction becomes more prominent. In addition, some reducing equivalents such as redox couples and enzymes are shifted

to their oxidized states under higher oxygen concentrations, with the effect that available reducing agents for the reduction of nitroxides become limited in the cellular environment. This also leads to a decrease in the rate of formation of hydroxylamines *in vivo*.[11,12]

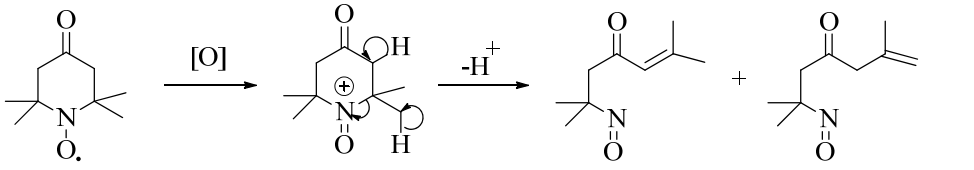
1.3.2 Oxidation

Nitroxide radicals are capable of undergoing a one electron oxidation to form oxoammonium salts (Scheme 1.6).[4]



Scheme 1.6 Oxidation scheme of a nitroxide

The stability of the oxoammonium salt is mainly dependant on the presence of other functional groups on the nitroxide unit. As an example, the oxammonium cation **1.20** (Scheme 1.7) of TEMPONE **1.19** spontaneously decomposes, owing to the presence of a ketone in the structure.[4,13]

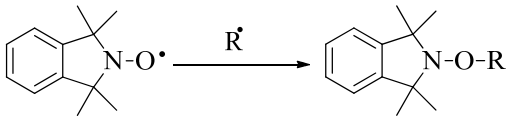


Scheme 1.7 Decomposition of the TEMPONE oxoammonium ion[4,13]

Usually the oxidation potential of nitroxides is relatively high (+0.21 to +1.09 V)[4], thus only strong oxidants such as chlorine, bromine or acids such as anhydrous HCl or Lewis acids such as aluminium halides can perform this oxidation.[4]

1.3.3 Radical trapping

Nitroxides are capable of scavenging carbon-centred radicals at near-diffusion controlled rates (10^7-$10^9 M^{-1}$ s^{-1}) to give stable diamagnetic alkoxyamines (Sheme 1.8).[14,15] This rapid scavenging or trapping of alkyl radicals is a widely known property of nitroxides and has been applied industrially in radical stabilizations, as well as for radical polymerization reactions.[16-18] In addition, this radical trapping property of nitroxides has been recently utilised as a novel protecting group methodology for syntheses involving nitroxides as precursors.[19]



1.15 **1.23**

Scheme 1.8 Scavenging of an alkyl radical by TMIO.

This trapping of alkyl radicals by nitroxides is less significant under normal biological conditions when they are applied as antioxidants or probe molecules due to extremely low concentrations of alkyl radicals in these systems. At higher molecular oxygen concentrations in biological systems, these alkyl radicals react with molecular oxygen to form oxygen-centred peroxide radicals. The reaction between oxygen-centred peroxide radicals and nitroxides is far less common,[5] since any gain in energy arising from recombination of these radicals cannot compensate for the loss of delocalization energy for the nitroxide group.[13] These stability and chemical/physical properties explain why nitroxides are applied in a wide variety of fields including controlled free radical polymerization, biological and material based antioxidants, etc.

1.4 APPLICATIONS OF NITROXIDES

Nitroxides have been used in a number of applications, including as antioxidants, spin traps, and as control agents in polymerization reactions.

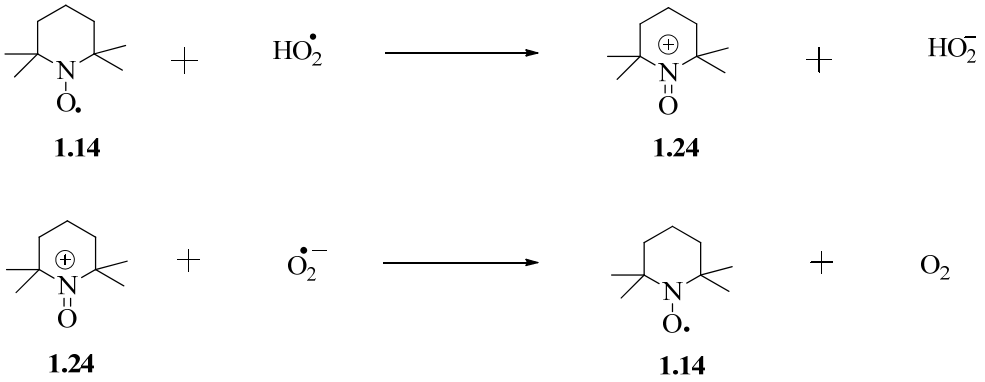
1.4.1 Antioxidants

Pro-oxidant species are chemical species that inhibit antioxidant systems in biological systems. Pro-oxidant species can be either reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, hydroxyl, peroxyl, alkoxyl radicals or reactive nitrogen species (RNS) such as nitric oxide, nitrogen dioxide, dinitrogen trioxide.

When the antioxidant level is lower than the pro-oxidant level in a biological system, the system is under a biological imbalance which is normally referred to as oxidative stress. Under these conditions, most of the important macromolecules of the system, such as lipids, DNA, and proteins can potentially be damaged.[20] This situation leads to several well-known disorders in biological systems, such as cancer,[21] ischemia-reperfusion injury,[22] Alzheimer's disease,[23] Parkinson's disease,[23] and aging.[23] To combat the symptoms derived from this radical damage, antioxidant levels of the system increase and it is at this stage that nitroxide radicals could play a positive role to support the organism. Features such as low toxicity, higher stability, redox and radical trapping properties of nitroxides have made them ideal candidates to act as antioxidants in biological systems. As an antioxidant, nitroxides have the ability to shuttle between reduced and oxidized states. While they shuttle between these two states, this shuttling process provides the reducing equivalents required for the detoxification of ROS/RNS, which causes a decrease within the pro-oxidant level of the cell. One of the best examples of this detoxification is the catalytic dismutation of superoxide by TEMPO. In this thoroughly investigated mechanism, one equivalent

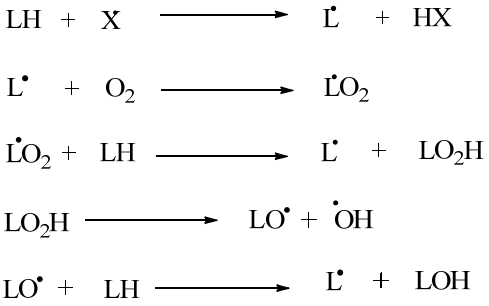
of TEMPO detoxifies two equivalents of superoxide via a nitroxide-oxoammonium ion couple.[24-26] (Scheme 1.9)

Nitroxides can also inhibit the lipid peroxidation in the lipid bilayer of the cell membrane owing to their redox shuttling ability. Most of the time, the initiators of lipid peroxidation are reactive oxygen species such as hydroxyl radicals. During the initiation step, a hydrogen atom is abstracted from a lipid molecule and a carbon-centred radical is formed. This carbon centred radical will react with molecular oxygen under aerobic conditions in order to form peroxyl radicals.[27] These peroxyl radicals then act as chain carriers[27] and abstract hydrogen from neighbouring lipid chains, serving to autocatalyse the free radical chain reaction (Scheme 1.10).



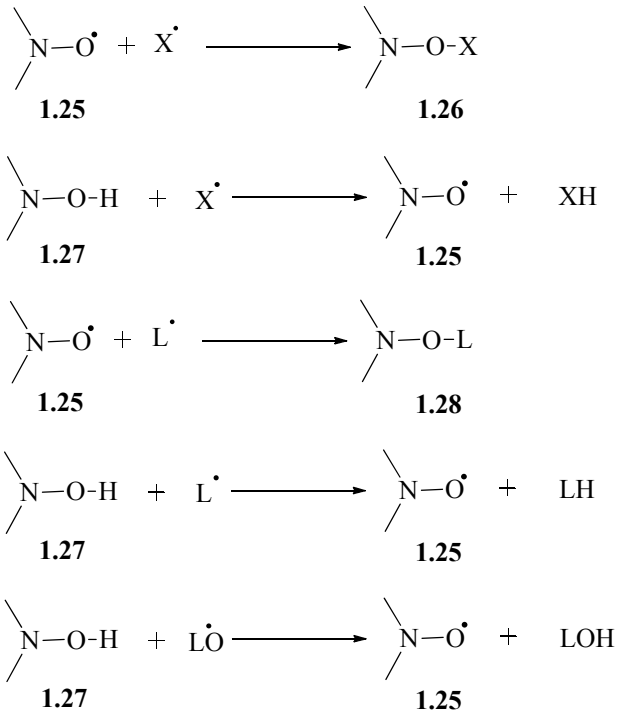
Scheme 1.9 Dismutation of superoxide radical by TEMPO (**1.14**)

Since nitroxides are capable of shuttling between the nitroxide and its reduced state, the initiator radical and some chain carrying radicals are inhibited,[28,29] as shown in Scheme 1.10 below.



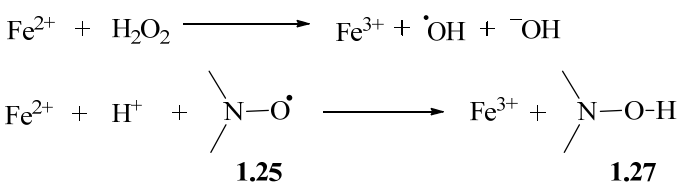
Scheme 1.10 Lipid peroxidation pathway.

According to Fenton chemistry, hydrogen peroxide produces highly reactive hydroxyl radicals (one type of ROS) by reacting with transition metals like Fe (II). This production is said to be one of the major pathways of producing hydroxyl radicals in biological systems which ultimately leads to oxidative stress.[20]



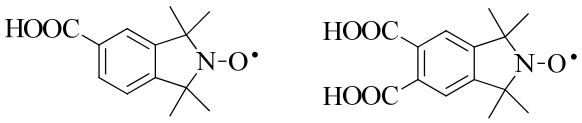
Scheme 1.11 Inhibition of lipid peroxidation by nitroxides.[28]

Nitroxides are capable of preventing the production of hydroxyl radicals by oxidizing Fe (II) ions before they interact with hydrogen peroxide (Scheme 1.12).[30,31] Redox interactions with metal ions have also been shown to enhance the catalase–like activity of hemeproteins such as metmyoglobin which facilitates the dismutation of hydrogen peroxide.[32]



Scheme 1.12 Hydroxyl radical formation by Fenton chemistry and the prevention of that by a nitroxide.

Recently it was found that isoindoline nitroxides are capable of reducing oxidative stress in cells affected with a neurodegenerative disease called *Ataxia telangiectasia* (A-T).[23] However, the applications of isoindoline nitroxides in this field have been limited due to the lack of water-solubilising structures. To date, the most effective isoindoline nitroxide synthesized to reduce the levels of oxidative stress in cells affected with A-T is 5-carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl (CTMIO) **1.29** (Figure 1.2) which has been used as an antioxidant successfully in an A-T mouse model. DCTMIO (**1.30**, Figure 1.2), which is 5,6-dicarboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl, has shown similar results to CTMIO. Some alternative synthetic approaches[33a] have also been introduced to synthesise CTMIO with comparable overall yields to the original synthesis.[33b]



CTMIO **1.29** DCTMIO **1.30**

Figure 1.2 Structures of stable isoindoline nitroxides

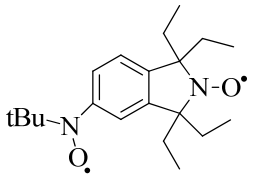
Since nitroxides, hydroxylamines and oxoammonium ions have redox cycling properties, they prevent the formation of pro-oxidant species through a broad range of antioxidant mechanisms. Therefore, derivatives of these compounds are utilized in both *in vivo* and *in vitro* in order to decrease the levels of oxidative stress.

1.4.2 Spin traps

The preparation of stable nitroxide radicals has received considerable attention, due to their multiple applications, including their use as spin traps. The spin trapping method is useful in investigating organic free radical processes qualitatively and quantitatively.[34] Nitroxides have also been widely used as spin traps in the study of

biological systems by ESR spectroscopy, as well as the study of the mechanism of free-radical polymerization.[35] This work has led to the design and synthesis of a stable nitroxide, 1,1,3,3-tetramethylisoindolin-2-yloxyl (TMIO), which efficiently and effectively scavenges carbon-centred radicals to produce stable, diamagnetic alkoxyamine products (Scheme 1.8).[35,36] A.H. White and co-workers identified the molecular structure and functions of TMIO by employing it as a spin trap.[36]

Rassat and co-workers have synthesized an isoindoline based nitroxide diradical as a *spin-labelled spin-trap* in order to detect nitric oxide (NO). Non-aromatic radicals such as 3-carbamoyl-2,2,5,5-tetramethyl-3-pyrrolin-1-yloxyl radical are reported to be unchanged in the presence of NO, while aromatic nitroxides like diphenylnitroxide and derivatives[37] or dihydroquinoline nitroxides react with NO.[38] Thus, they have brought these two features together by combining an aromatic nitroxide and pyrroline nitroxide on a isoindoline backbone, and synthesized a new diradical, as shown in Figure 1.3 below.

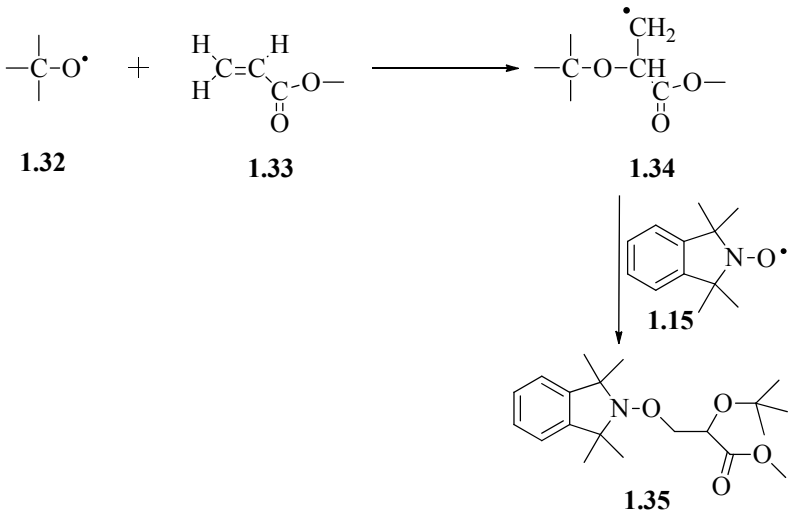


1.31

Figure 1.3 Structure of isoindoline based diradical

They then bubbled nitric oxide in a deoxygenated solution of diradical in ethanol, and a sample withdrawn from the mixture was analysed by EPR spectroscopy and compared with the EPR spectrum of the diradical. From the comparison, they showed that a single broad line of the diradical has turned to a typical 3-line EPR spectrum of a nitroxide.[39] From this experiment, they clearly showed that nitroxides are capable of trapping radicals. However, the use of this spin trap in order to scavenge NO radicals in biological systems can be problematic, due to the reduction

of the nitroxide moieties of the diradical by ascorbic acid and other endogenous cellular reductants.



Scheme 1.13 Carbon-centred radical, derived from the "head" addition of *tert*-butoxy radical **1.32** to methylacrylate **1.33**, trapped by TMIO[40]

Griffiths and co-workers reported some previously unobserved reaction pathways for the attack of *tert*-butoxy radicals on vinyl monomers by exploiting the radical scavenging feature of stable nitroxides.[40] To develop this experiment, they required an efficient separation of alkoxyamine products in sufficient quantities in order to determine their structures by conventional spectroscopic methods. For that, reverse-phase High Pressure Liquid Chromatography (HPLC) was used with the aid of UV detection in order to analyse the complex mixtures obtained from the experiment. However, most of the adducts obtained by this experiment from readily available TEMPO (Figure 1.1) only showed UV absorbance in the region where HPLC solvents also absorb. Thus TMIO (Figure 1.1) which possesses a UV chromophore was employed as the radical scavenging nitroxide. This trap allowed these workers to examine the multiple reaction pathways that occur with free radicals.[40] In these experiments, *tert*-butoxy radicals in methyl acrylate containing TMIO were shown to

react by several competing pathways such as "head" addition (**1.34**, Scheme 1.13), "tail" addition, H-abstraction and fragmentation.[40]

All the initially formed carbon-centred radicals (such as **1.34**) from the multiple competing pathways were efficiently scavenged by TMIO (**1.15**) due to its high trapping rate, therefore no oligomers or polymers are formed. However, another reaction that carbon-centred radical **1.34** could undergo was dimerization, but the dimer was not shown (in scheme 1.13) as **1.34** could have been scavenged by TMIO efficiently.[40] Thus, the technique developed by employing TMIO (**1.15**) as a spin trap provides valuable insight into the selectivity of attack of *tert*-butoxy radicals on vinyl monomers, and demonstrate that "tail" addition is the more dominant process.

1.4.3 Initiators in Nitroxide-mediated polymerization

Living free radical polymerization, which is also known more correctly as controlled free radical polymerization, attracted wide interest among polymer chemists, as it circumvents some of the limitations arising with conventional free radical polymerization. Conventional polymerization produces a broad range of molecular weight distributions. Frequent termination reactions occur during conventional polymerization, which leads to a distribution of molecular weight. Controlled polymerization using nitroxides, on the other hand provides narrow polydispersity homopolymers with high purity and end group functionality.[41]

There are several types of controlled free radical polymerization, such as atom transfer radical polymerization (ATRP),[42-44] nitroxide-mediated living radical polymerization (NMP),[45,46] and reversible addition-fragmentation chain transfer polymerization (RAFT).[47,48] Among these three, nitroxide-mediated free radical polymerization has been widely used for controlled free radical polymerization for the longest time. In the mid 1980s, Rizzardo and co-workers demonstrated the first

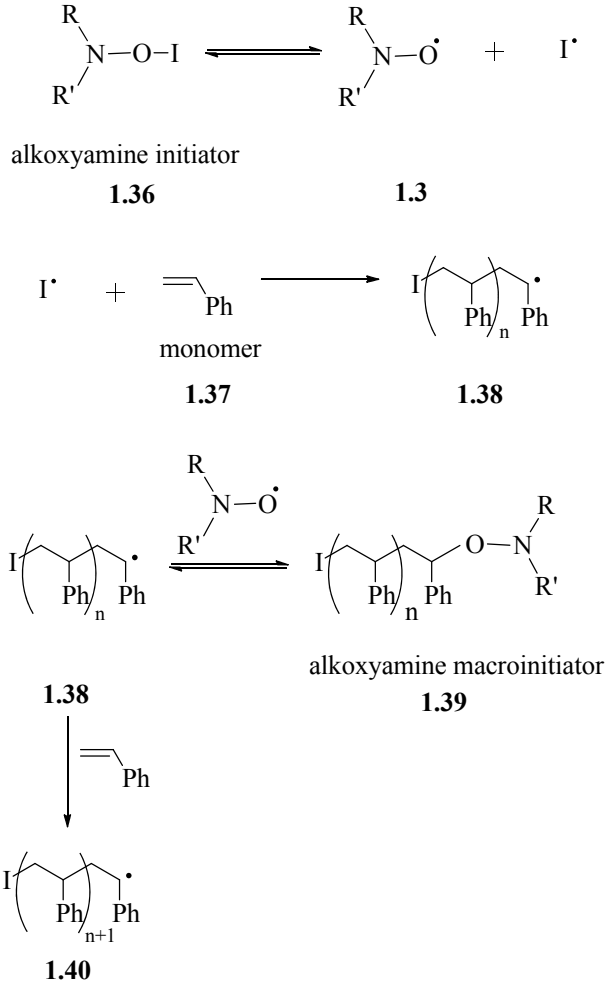
successful NMP by synthesizing polystyrene using TEMPO (**1.14**) as the controlling agent.[45] After this successful controlled polymerization, different types of nitroxides and alkoxyamines have been synthesized as controlling agents for NMP.[45,49]

NMP can be carried out by generating the alkoxyamine *in situ*; that is, by adding a free radical initiator to a solution containing the nitroxide and monomer, or by using a pre-prepared alkoxyamine initiator. The efficiency of the alkoxyamine as an initiator depends on the rate of decomposition of the alkoxyamine initiator and the alkoxyamine macroinitiator.[50] It is well known that the rate contant of the homolysis of C-O bond of the alkoxyamine initiator depends on both the structures of the nitroxide and the carbon centred radical.[51-54]

Successful nitroxide-mediated living radical polymerization requires an alkoxyamine initiator that dissociates to a carbon centred radical (I) and a nitroxide. The former then initiates the growth of the polymer by adding it to a monomer and the latter forms a new alkoxyamine called alkoxyamine macroinitiator, with a newly formed monomer radical. This alkoxyamine macroinitiator should dissociate quickly in order to continue the growth of the polymer in a reasonable time by further monomer addition, while simultaneously recombining with the nitroxide rapidly to minimise any chances of bimolecular termination reactions (Scheme 1.14).

The dependence of the rate of homolysis of C-O bond on the structure of the nitroxide is mainly due to the steric factors, but thermodynamic, electronic and polar factors are also of secondary importance.[55] Rizzardo and co-workers have carried out some molecular orbital calculations for the C-O bond homolysis of a series of alkoxyamines and showed that the structure of the alkoxyamine determines the strength of the C-O bond.[56] In addition, Kazamaier *et al.* reported that the bond dissociation energy of the C-O bond of the alkoxyamine formed with di-*tert*-butyl

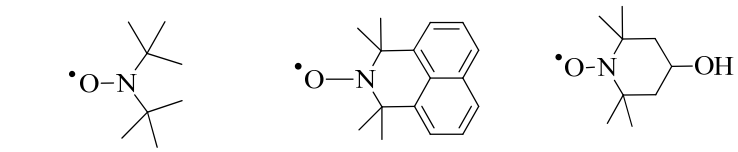
nitroxide **1.41** (Figure 1.4) is lower compared to that of TEMPO (**1.14**, Figure 1.1), due to the higher steric crowding possessed by di-*tert*-butyl nitroxide **1.41**.[57] It was predicted that an alkoxyamine based on a 7-membered ring nitroxide may show an enhanced homolysis rate over a similarly substituted smaller ring or open chain nitroxides.[55]



Scheme 1.14 Proposed mechanism for nitroxide-mediated polymerisation of styrene.[46,55]

One method that has been used to reduce the stability of nitroxides and make them ideal candidates in NMP is to introduce bulky functional groups around the nitroxide moiety. Such a nitroxide will make an alkoxyamine which dissociates at much faster rate during the polymerization process. The best example of this type of nitroxide is TEIO (**1.13**, Figure 1.1), which gave much faster homolysis of C-O bond during the

polymerization of styrene and methyl methacrylates compared to the less hindered methyl analogue, TMIO (**1.15**, Figure 1.1).[58] Another novel nitroxide, TMAO (**1.42**, Figure 1.4) has been synthesized to use as a control agent in NMP. TMAO (**1.42**) possesses structural similarities to the isoindoline class of nitroxides (most notably structural rigidity) and similarities to the TEMPO class of nitroxides (in terms of ring size) which indicate it should be an ideal candidate for the controlled polymerization of styrene.[58]

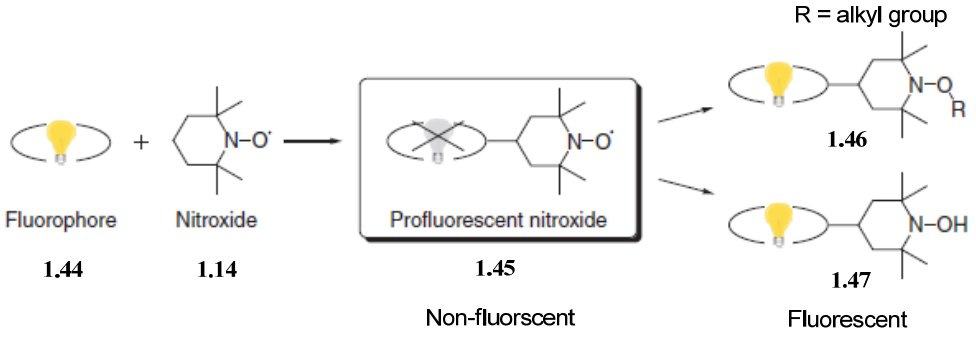


di-*tert*-butyl nitroxide **1.41** TMAO **1.42** **1.43**

Figure 1.4 Structures of ideal control agents in NMP.[45,58]

1.4.4 Profluorescent probes

One of the most important and recent application of nitroxides is their use as profluorescent probes. In this case, nitroxides are covalently tethered to a fluorophore which leads to the suppression of fluorescence. Removal of the unpaired electron of the nitroxide allows the molecule to regain its natural fluorescence. These fluorophore-nitroxide linked species are referred to as "profluorescent nitroxides" (Scheme 1.15).



Scheme 1.15 Cartoon representation of the activity of profluorescent nitroxides.[59]

In the normal process of fluorescence, a fluorophore in the singlet ground state (S_0) is excited to the first singlet excited state (S_1) by absorbing a photon (Figure 1.5). This excited fluorophore will return to the singlet ground state again in two steps. During the first step, some of the energy of the excited fluorophore will be dissipated to the surrounding by vibrational relaxations (VR) or by internal conversions (IC) until the fluorophore is returned to the lowest vibrational level of S_1. The path of the second step mainly depends on the energy gap between S_1 and S_0. When the energy difference between S_1 and S_0 is small, this excited fluorophore is most likely to emit its energy via internal conversion in a non-radiative fashion. If there is an appreciable energy difference between S_1 and S_0, on the other hand, the excited fluorophore will return to ground state S_0 either by emitting energy in the form of a photon (a process called "fluorescence") or the multiplicity of the excited electron will be converted to the excited triplet state by a process called intersystem crossing (ISC). Further deactivation of the excited triplet-state fluorophore can occur by the emission of a photon (phosphorescence), or through thermal interactions.

The mechanism of fluorescence suppression of profluorescent nitroxides is most likely to arise through electron-exchange interactions between the paramagnetic nitroxide and the excited state fluorophore.[60] When the unpaired electron of the nitroxide interacts with the electrons of the fluorophore, the multiplicities of these electrons change. As a result, the singlet ground state S_0, and first excited singlet state S_1, are present as doublet states D_0 and D_n, respectively, due to coupling with the antiparallel spin of the nitroxide. The excited triplet state T_1, becomes the lowest excited doublet state (D_1). Now all the previous forbidden transitions such as inter-system crossing and T_1 to S_0 energy loss are spin allowed. This brings the total

fluorescence quantum yield to a lower level due to the increased rates of ISC, and results in the significant suppression of the fluorescence.[60]

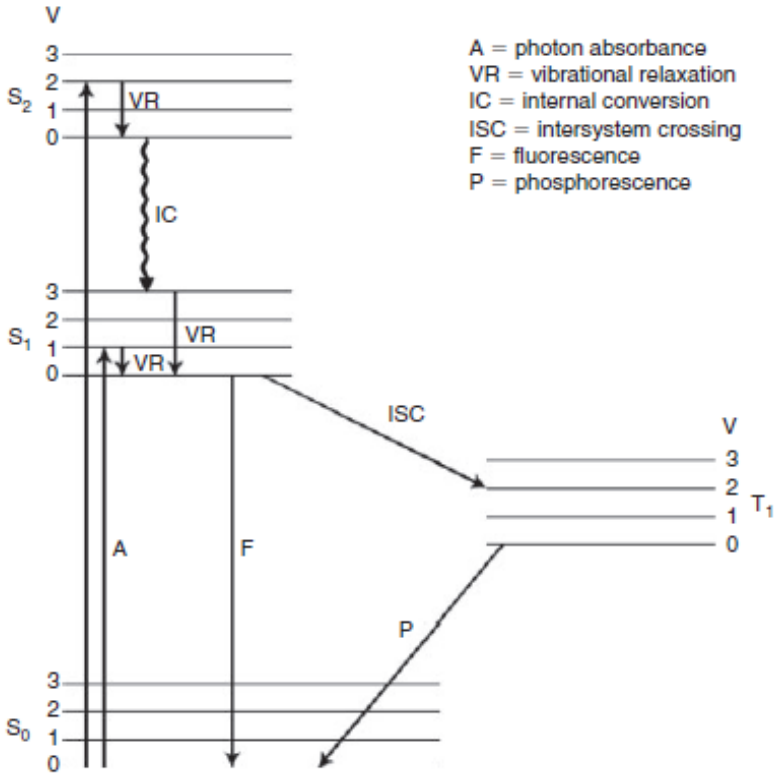
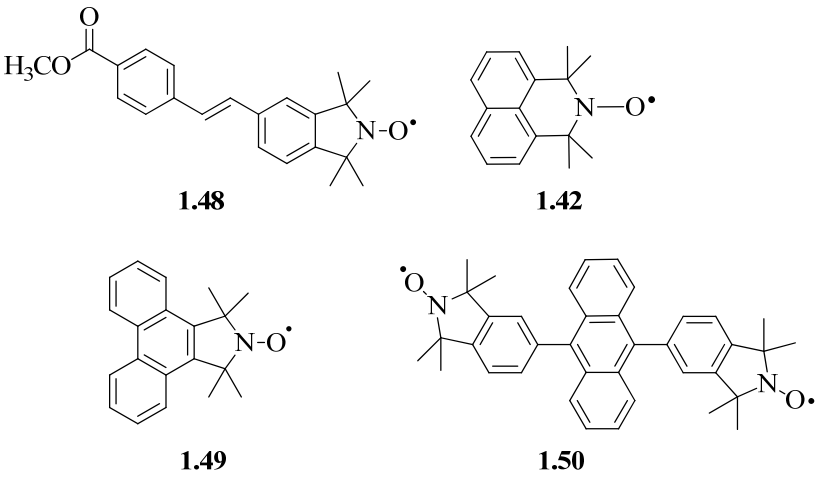


Figure 1.5 Jablonski diagram of fluorescence and phosphorescence of an excited electron.[59]

This suppression of fluorescence in the presence of a nitroxide radical and the return of the fluorescence emission by reduction or radical trapping of the nitroxide have been demonstrated in various studies by Blough[61,62] and Scaiano.[63,64] This special feature of nitroxide-fluorophore couples has been used by chemists in order to investigate a number of free radical processes. Some examples of nitroxide-fluorophore couples are shown in Figure 1.6 below.



1.48 **1.42**

1.49 **1.50**

Figure 1.6 Some stable profluorescent nitroxides, **1.48**[65], **1.42**[66], **1.49**[67] and **1.50**[68]

The novel profluorescent nitroxide, 1,1,3,3-tetramethyldibenzo[*e*,*g*]isoindolin-2-yloxyl (TMDBIO, **1.49**, Figure 1.6) which contains a phenanthrene fluorophore covalently attached to a five-membered ring nitroxide has been utilized as a sensitive probe in monitoring thermo-oxidative degradation of polypropylene.[69] During the induction period of this degradation, the free radicals formed are detected by TMDBIO (**1.49**) using spectrofluorimetry. However the other nitroxide probes shown in Figure 1.6 are also under investigation as tools in imaging the degradation of polypropylene and other polymer systems. In recent experiments, it was found that profluorescent nitroxides are capable of probing the induction period of polymer oxidation by measuring the concentration of alkyl radicals produced.[70] On scavenging the alkyl radicals produced during the polymer oxidation process by the profluorscent nitroxide, chemiluminiscence is returned. Therefore, this property has been used to quantify the concentration of alkyl radicals in terms of radical scavenging efficiency of the PFN.[70]

1.4.5 Spin labels

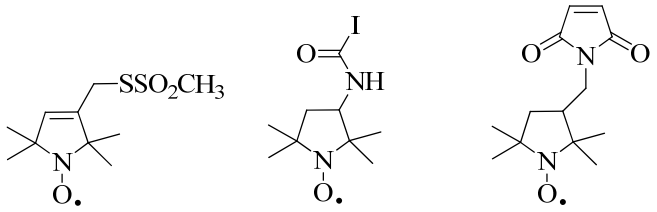
The electron paramagnetic resonance (EPR) spectrum of a nitroxide radical is sensitive to the polarity of the surrounding microenvironment of the nitroxide and its

molecular motions.[71a] This characteristic feature can be used to investigate the conformational and structural information of important biological molecules by attachment of the nitroxide and analysing EPR spectral characteristics. The function of the nitroxide in this special process is referred to as "spin labelling".

This technique was first demonstrated by McConnell and co-workers by attaching a PROXYL nitroxide to poly-L-lysine.[71a,71b] When the pH of the surrounding environment is acidic, poly-L-lysine adopts a coil configuration and the molecular motion of the nitroxide is therefore enhanced. This produces a narrow linewidth in the EPR spectrum. In a high pH environment, poly-L-lysine adopts an alpha-helix, which leads to broad linewidth in the EPR spectrum, due to the restriction of the molecular motion of the nitroxide.[71a] In the same way, the conformational properties of bovine serum albumin (BSA) have been investigated.[71b] Sigurdsson and co-workers recently synthesized nucleoside spin-labelled isoindoline based nitroxides and incorporated into DNA.[72] From this synthesis, it was found that the mobility of the spin label was limited due to the formation of intramolecular H bonding between the spin label and DNA.[72] This demonstrates a versatile feature of nitroxide spin labels which is likely to continue to be an active area of research in the near future.

McConnel first reported a form of this spin labelling technique in 1965. This has subsequently been improved to provide a powerful analytical tool which is called "site-directed spin labelling" (SDSL). In this technique, nitroxides are covalently bound to a specific site of the biomolecule, and conformational and structural characteristics of this particular region are studied. This method is well suited and widely used to study small soluble proteins, membrane proteins, and large protein complexes. Another reason for the growing popularity of this SDSL technique is its applicability to study large 'solid-like' macromolecular complexes. In addition,

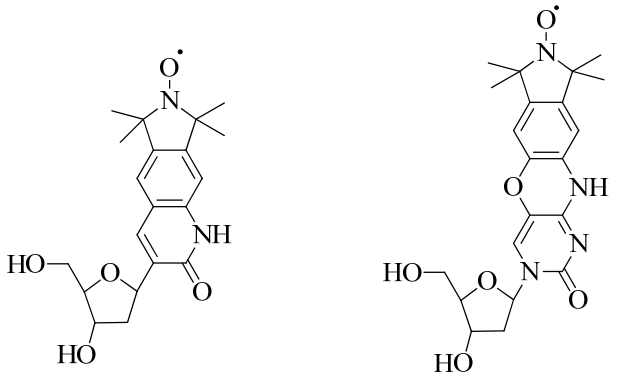
SDSL is a relatively inexpensive and sensitive technique, requiring only picomole-sized samples for analysis.[73] In the characterization of protein structure, a native amino acid in the protein is substituted for cysteine *via* site-directed mutagenesis and the resultant modified protein is subsequently derivatised at the cysteine residue with a sulfhydryl-reactive nitroxide spin label. Some of the common nitroxides used for this purpose are shown in Figure 1.7



1.51 **1.52** **1.53**

Figure 1.7 Nitroxide spin probes used in site directed spin labelling of proteins.[73]

Recently, Sigurdsson and co-workers have reported several site-directed spin labelling methods for nucleic acids, enabling linking of nitroxides to specific nucleotides, either to the base, the sugar or the phosphate.[74] These workers focussed on three labelling strategies: spin labelling during chemical synthesis of nucleic acids, post-synthetic labelling and non-covalent labelling. This work is likely to continue to be an active area of research in the near future.



1.54 **1.55**

Figure 1.8 Isoindoline nitroxides as rigid spin labels incorporated with nucleosides.[74]

Apart from these main applications of nitroxides, some other applications including as probes for EPR spectrometry,[75] potential redox mediators for dye-sensitised solar cells,[76] contrast enhancing agents[8] for magnetic resonance imaging applications, and as molecular units[77] in the synthesis of molecular magnetic materials, are of notable importance. Due to the wide range of applications possible for nitroxides, the interest in this field continues to grow. As a result, several different structural classes of nitroxides have been synthesized by chemists, including nitronyl nitroxides, isoindoline nitroxides, pyrrolidine nitroxides, piperidine nitroxides, pyrroline nitroxides, nitroxides annulated heterocycles etc. Of these species, isoindoline nitroxides and related heterocyclic nitroxides are the focus of this project.

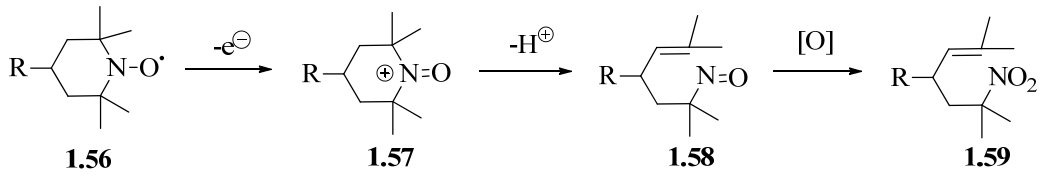
1.5 ISOINDOLINE NITROXIDES

The chemistry and utilization of nitroxides are regularly reviewed from the beginning[78,79] by the scientists active in this field; thus several excellent reviews[80-82] and monographs have been published on nitroxides in the past 15 years.[83-85] One class of nitroxides called isoindoline nitroxides possess several advantages over many of the other available nitroxides such as piperidine- and pyrrolidine- based nitroxides.

1.5.1 Special properties of isoindoline nitroxides

The structural importance of isoindoline nitroxides derives from the fused aromatic ring, which provides rigidity to the structure and gives resistance towards ring opening reactions that are the main decomposition pathway of pyrrolidine and piperidine nitroxides (Scheme 1.16).[86] Enhanced thermal and chemical stability shown by isoindoline nitroxides (such as unreactivity towards olefins, relative inertness towards free radical attack) in a wide variety of chemical environments

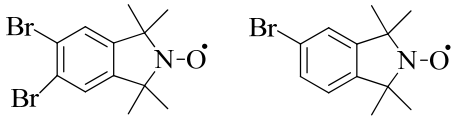
including acidic and basic solutions[87] also arises from the fused aromatic ring and lack of abstractable hydrogen atoms in the structure.[40] Additionally, the fused ring acts as a UV absorbing chromophore, so these isoindoline structures can be detected in high pressure liquid chromatography (HPLC) using UV absorption.[40] The symmetrical nature of isoindolines such as TMIO (**1.15**) and TEIO (**1.13**) generates a minimum number of isomers in the radical adducts, thus facilitating structural analysis due to fewer signals in the NMR spectra.[40]



1.56 **1.57** **1.58** **1.59**

Scheme 1.16 Ring-opening reactions of TEMPO based monocyclic nitroxides.[86]

Preliminary studies indicated that isoindoline nitroxides possess isotropic EPR linewidths which are much smaller than the pyrrolidine and piperidine nitroxides,[87] therefore this feature can be used as a characteristic identification tool for these compounds, and also leads to increased accuracy in EPR oximetry.[68] In addition, isoindoline nitroxides are used as intermediates in the synthesis of more complex structures that have a variety of applications.[88] Substitution of the aromatic ring provides a range of different compounds[87a,87b] (Figure 1.9) with significantly different physical properties, due to the loss of the symmetry of the substituted nitroxide and associated influences on the electron density in the ring.



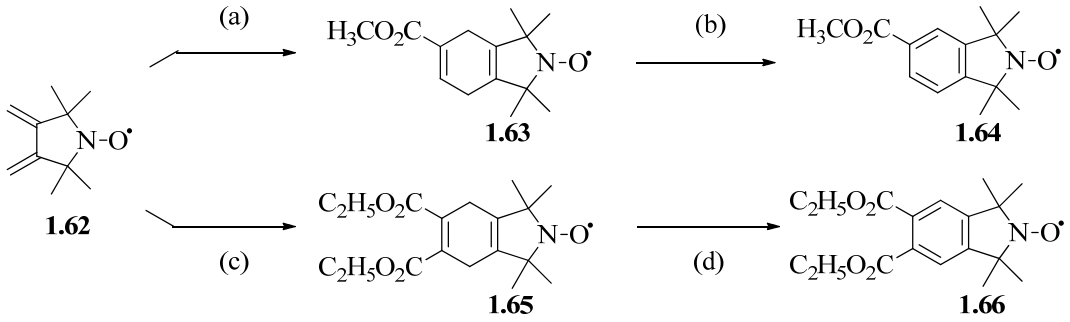
1.60 **1.61**

Figure 1.9 Bromo-substituted isoindoline nitroxides (**1.60** and **1.61**) with noval physical properties [87a]

Owing to the advantages and special properties of isoindoline nitroxides over the commercially available nitroxides, it is important to investigate more efficient means to synthesize isoindoline nitroxides in higher yield. Therefore, it is appropriate to investigate some of the special synthetic procedures used by the chemists to synthesize isoindoline nitroxides with the yields obtained.

1.5.2 Different synthetic approaches to isoindoline nitroxides

There are three main approaches described for the synthesis of isoindoline nitroxides. Hideg and co-workers have reported a synthetic approach for isoindoline nitroxides *via* Diels-Alder reaction (Scheme 1.17) of paramagnetic symmetric pyrrolidine diene **1.62** with methyl propynoate or diethyl acetylenedicarboxylate to give 5-substituted (**1.64**) or 5,6-disubstituted isoindoline nitroxides (**1.66**), respectively, following oxidative aromatization.[89]



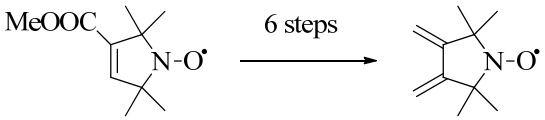
Scheme 1.17 Isoindoline nitroxide synthesis by Diels-Alder reaction.[89]

Reagents and conditions: (a) $HC{\equiv}CCO_2Me$ (1.0 equiv)/toluene, 110 °C, 2-4 h (62-77%); (b) activated MnO_2 (10 equiv)/$CHCl_3$, 61 °C, 8 h (58%); (c) $EtO_2CC{\equiv}CCO_2Et$ (1.0 equiv)/toluene, 110 °C, 2-4 h (62-77%); (d) DDQ (1.0 equiv)/toluene, 110 °C, 2 h (35%)[89]

Heating diene **1.62** with methyl propynoate and diethyl acetylenedicarboxylate in benzene gave the Diels-Alder adducts **1.63** and **1.65**. Aromatization of compound **1.63** with activated MnO_2[90] gave 5-monosubstituted isoindoline nitroxide **1.64**, while compound **1.65** could be aromatized by reflux with 2,3-dichloro-5,6-dicyano-1,4-

benzoquinone (DDQ)[91] to give the 5,6-disubstituted isoindoline nitroxide **1.66**.[89] However, a synthesis of a similar aromatic substituted isoindoline nitroxide was reported by Micallef and co-workers in 1998 through a different synthetic route[92] which will be discussed later.

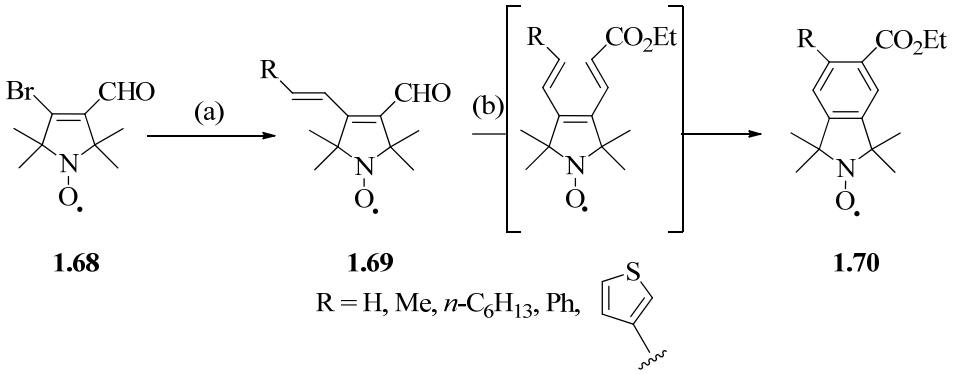
The synthesis of paramagnetic diene nitroxide **1.62** was challenging, but it was expected to be a versatile intermediate and it was therefore pursued and achieved starting from the commercially available nitroxide **1.67**[93] in six steps (Scheme 1.18).[89,93] When the yields of the substituted isoindoline nitroxides **1.64** and **1.66** were calculated starting from commercially available precursor **1.67**, the overall yield was less than 5%, which is not viable for large-scale synthetic production. Thus, alternative synthetic procedures for the synthesis of these important isoindoline nitroxides need to be developed.



1.67 **1.62**

Scheme 1.18 Synthetic route of diene **1.62** from commercially available **1.67**.[89,93]

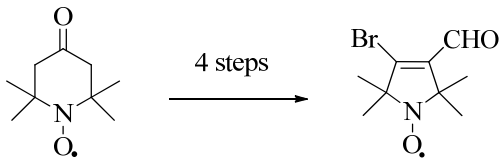
For the construction of six-membered ring nitroxides, not only cycloaddition reactions (as in the previous example), but also electrocyclic reactions can be used. The elecrocyclic reaction of 1,3,5-hexatrienes provides a six-membered ring which can be dehydrogenated to produce an aromatic ring. This 6π-electrocyclization is involved in the biosynthesis of vitamin D,[94] and this process is often used for the synthesis of complex organic molecules like 3-nitroindoles,[95] and natural products such as coralydine[96] and hyellazole.[97] In 2009, Hideg and co-workers showed that electrocyclization reactions can also be used to synthesize isoindoline nitroxides.



Scheme 1.19 Synthetic route of isoindoline nitroxides by electrocyclic reactions.[98]

Reagents and conditions:(a)RHC=CB$(OH)_2$(1.1 equiv), $Pd(PPh_3)_4$ (0.03 equiv), 10% aquous Na_2CO_3, dioxane, reflux (N_2), 3 h, 55-73%; (b) 1. NaH (1.2 equiv), $(EtO)_2P(O)CH_2CO_2Et$ (1.2 equiv), 0 °C, 30 min, then **1.69** (1.0 equiv), reflux, 5 h; 2. MnO_2 (2.0 equiv), reflux, 30 min, 78-85%.[98]

According to the synthetic route shown in Scheme 1.19, the Suzuki-Miyaura reaction[99] of **1.68** with 2-substituted vinylboronic acid under the given experimental conditions in step (a) gave a series of aldehydes **1.69** with all the given R-groups below. This series of aldehydes was reacted with triethyl phosphonoacetate in toluene in the presence of sodium hydride to give 1,3,5-trienes.[100] The trienes were not isolated at this point, but instead the crude product obtained was reacted with MnO_2 under the experimental conditions given below (Scheme 1.19) to produce 5,6-disubstituted isoindoline nitroxides **1.70** with five different R-groups. Starting material **1.68** is not commercially available and had to be synthesized from commercially available nitroxide, TEMPONE (**1.19**) over four steps (Scheme 1.20). The yield for synthesizing **1.68** starting from **1.19** was 18%. [101,102]

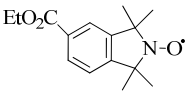
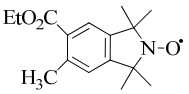


1.19 **1.68**

Scheme 1.20 Synthesis of aldehyde **1.68** from commercially available **1.19**[101,102]

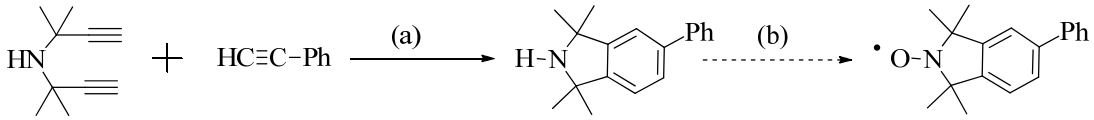
The utility of the electrocyclic reactions over the Diels-Alder reactions in synthesizing isoindoline nitroxides is demonstrated through comparison of the overall yields obtained for the synthesis of two isoindoline nitroxides, **1.70a** and **1.70b**, starting from commercially available **1.67** and **1.19** (Table 1.1). Although electrocyclic reactions are more succesfull than Diels-Alder reactions, as shown in Table 1.1 below, neither are very efficient, and so there is clearly a need for a new synthetic method for the increased efficiency higher yield synthesis of these nitroxides.

Table 1.1 Synthetic yields of isoindoline nitroxides from two synthetic approaches.

Synthetic Methodology	**Isoindoline nitroxides**	
	 **1.70a**	 **1.70b**
Electrocyclic reaction	8.7%	8.0%
Diels-Alder reaction	3.0%	0.4%

In another approach, tetramethyldipropargylamine **1.71** was reacted with phenylacetylene **1.72** in the presence of catalyst, bis(acetonitrile)bis(diethylfumarate)cobalt(0) at 80 C under N_2 for 72 hours.[103] This reaction gave phenyl-substituted 1,1,3,3-tetramethylisoindoline **1.73** in 81% as determined by GLC, and an isolated yield of 73% by column chromatography.[103] The secondary amine **1.73** could be treated with $H_2O_2/Na_2WO_4.2H_2O/NaHCO_3$ in methanol[35] (step b, Scheme 1.21) to convert to the corresponding substituted TMIO (**1.74**) with a good yield, but the workers reported neither this conversion or the actual isolated yield of nitroxide **1.74** which could be obtained by this method. However, the synthesis of **1.73** has several drawbacks such as higher cost, if commercially available **1.71** is utilized in the reaction. On the other hand, the

synthesis of **1.71** in a laboratory is also time consuming (approximately 10 days) and low yielding.[104]

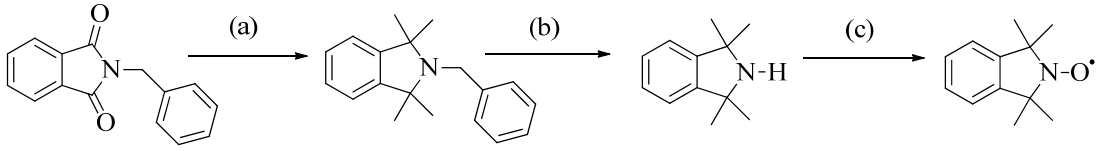


1.71 **1.72** **1.73** **1.74**

Scheme 1.21 Possible synthesis of substituted isoindoline nitroxide TMIO, **1.74** via cyclodimerization of **1.71** and **1.72**[103]

Reagents and conditions: (a) **1.71** (1.0 equiv), bis(acetonitrile)bis(diethylfumarate)cobalt(0) (0.03 equiv), acetonitrile, N_2, 80 °C, 72 h, 73%;[103] (b) **1.73** in MeOH/CH_3CN, then aqueous $NaHCO_3$ (1.0 equiv), $Na_2WO_4.2H_2O$ (0.25 equiv), H_2O_2 (30%, 4.0 equiv), room temperature, 32 h, 92%.[35]

The third synthetic approach to isoindoline nitroxides was developed by Griffiths and co-workers and represents the most commonly used synthetic procedure for the synthesis of these species. These workers synthesized TMIO by tetramethylation of *N*-benzylphthalimide using methylmagnesium iodide in refluxing toluene, followed by deprotection and oxidation.[35] The synthetic scheme of this procedure is shown below. (Scheme 1.22)



1.75 **1.76** **1.77** **1.15**

Scheme 1.22 Synthesis of isoindoline nitroxide (TMIO, **1.15**) via Grignard addition to *N*-benzylphthalimide **1.75**[35]

Reagents and conditions: (a) MeI (6.0 equiv), Mg (6.0 equiv), Et_2O, N_2, rt, then Toluene reflux, 4 h with **1.75** (1.0 equiv), 37%; (b) **1.76**, AcOH, H_2/Pd/C, 3 h, 60 lb/in^2, then aqueous NaOH (10%), Et_2O, 96%; (c) **1.77** in MeOH/CH_3CN, then aqueous $NaHCO_3$ (1.0 equiv), $Na_2WO_4.2H_2O$ (0.25 equiv), aqueous H_2O_2 (30%, 4.0 equiv), rt, 32 h, 92%.[35]

The tetraalkylation was first attempted by treating **1.75** with excess MeMgI in refluxing toluene for 4 hours. This reaction gave **1.76** as the major product (35-37%), along with a small amount (2-3%) of **1.82** (Figure 1.10), which was easily removed by recrystallization.[35] No other well-defined products were isolated from this reaction. Compound **1.75** is not commercially available, so it was synthesized from commercially available phthalic anhydride **1.84** (Scheme 1.23) in high yield (98%).[106] The overall synthetic yield of the isoindoline nitroxide, TMIO, calculated from the commercially available phthalic anhydride **1.84**, was much improved (32%) compared to the other reported methods.

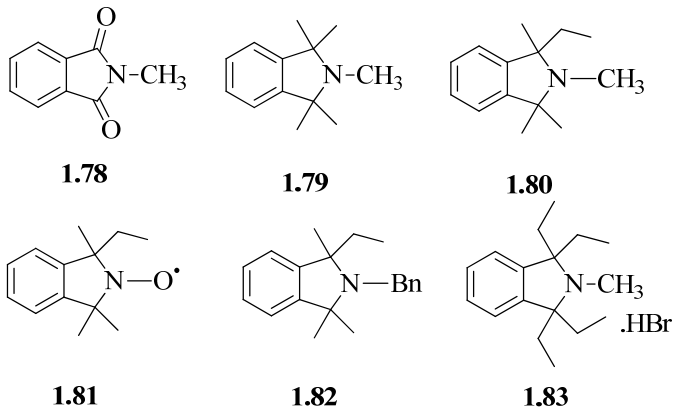
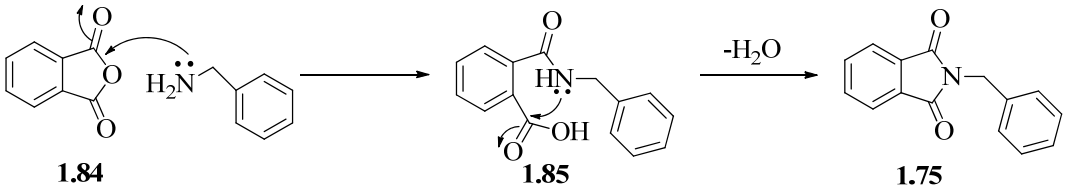


Figure 1.10 Structures observed in the synthesis of TMIO starting from **1.75** and **1.78** [35]

In a similar fashion, Caldararo and and co-workers synthesized the ethyl version of TMIO (abbreviated here as TEIO **1.13**, Figure 1.1) starting from *N*-benzylphthalimide **1.75** in a yield of 34%.[88] Comparing the yields obtained by the three synthetic approaches: 8-9% by electrocyclic reactions (6 steps), 1-3% by Diels-Alder reactions (8 steps), and 32-34% by Grignard addition on to phthalimide (4 steps): The Grignard methodology is clearly the most efficient. However, if the Grignard approach could be further improved by increasing the efficiency of the

individual steps involved, this would further increase the efficiency of the generation of these nitroxides.



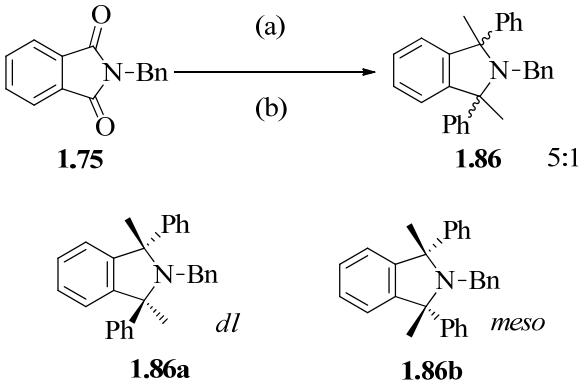
1.84 **1.85** **1.75**

Scheme 1.23 'Amino-de-acyloxy-substitution' mechanism.[107]

In the first step of this route (that is conversion of **1.84** to **1.75**), phthallic anhydride **1.84** was reacted with benzylamine in refluxing acetic acid for 2 hours to give **1.75** in 98% yield (after recrystallization).[106] The mechanistic process of forming **1.75** from **1.84** is referred to as 'amino-de-acyloxy-substitution' (Scheme 1.23).[107] This reaction occurs in near quantitative yield and therefore cannot be further improved.

However, if Scheme 1.22 is examined clearly, the most important and difficult step in this pathway is the Grignard tetraalkylation of **1.75**. Since this step involves a tetraalkylation, it requires a high temperature to obtain complete conversion. Thus this reaction was usually performed in refluxing toluene at 110 °C for 4 hours and produced a yield of 35-37% for **1.76**. Although Grignard reagents represent one of the most versatile tools in the organic synthetic chemistry, the nature of these reagents in solution and the mechanism of forming the products are still not completely understood. For this reaction, Braslau and co-workers investigated the mechanism of forming these tetraalkylated products by reacting **1.75** with 2 equivalents of phenylmagnesium bromide at 80 °C for 30 mins followed by the addition of 4 equivalents of methylmagnesium bromide and refluxing of the mixture at 110 °C for 12 hours. According to the products isolated, (including *meso*-

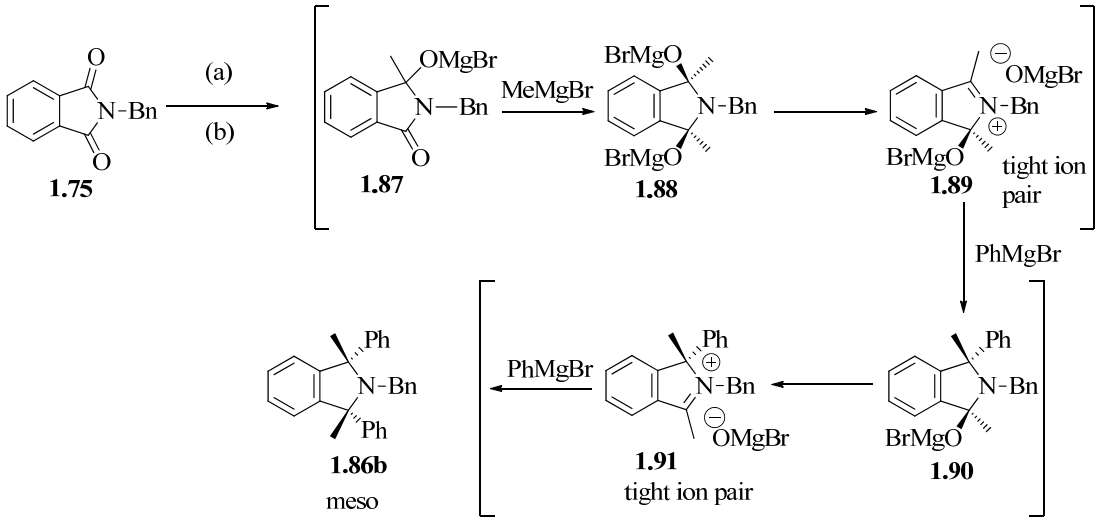
dimethyl-substituted amine **1.86b**, Scheme 1.24), they proposed a mechanism for the formation of the tetraalkylated product.[108]



Scheme 1.24 Formation of meso-dimethyl-substituted product **1.86b**.[108]

Reagents and conditions: (a) MeMgBr (2.0 equiv), Toluene, 80 °C, 30 mins. (b) PhMgBr (4.0 equiv), Toluene, 110 °C, 12 h[108]

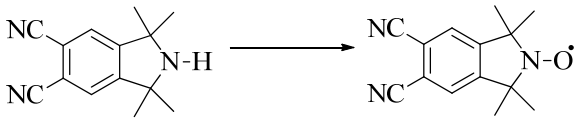
According to Scheme 1.25, the second equivalent of MeMgBr adds to the face opposite to the large alkoxy magnesium salt, forming intermediate **1.88**. Elimination of OMgBr gives iminium salt **1.89** as a tight ion pair. The first equivalent of PhMgBr adds to the face opposite to the remaining alkoxy magnesium bromide to give intermediate **1.90**, and the subsequent elimination of OMgBr gives intermediate **1.91** also as a tight ion pair. Addition of the second equivalent of PhMgBr occurs opposite to the large oxymagnesium bromide salt giving *meso* **1.86b** (Scheme 1.25).[108] This explanation provides a reasonable guide to understanding the unexplored stereochemistry of intermediates formed during the addition of Grignard reagents on *N*-benzylphthalimide **1.75**.



Scheme 1.25 Mechanism for the tetraalkylation of *N*-benzylphthalimide **1.75**.[108]

Reagents and conditions: (a) MeMgBr (2.0 equiv), Toluene, 80 °C, 30 mins. (b) PhMgBr (4.0 equiv), Toluene, 110 °C, 12 h[108]

The next step of this reaction is the deprotection of tetramethyl adduct **1.76** to give 1,1,3,3-tetramethylisoindoline **1.77**. One reason for choosing *N*-benzylphthalimide **1.75** for the synthesis of TMIO (**1.15**) is the ease of deprotection of the nitrogen. Griffiths and co-workers originally achieved this deprotection by simple hydrogenolysis. For that, *N*-benzyl-1,1,3,3-tetramethylisoindoline **1.76** was reacted with H_2 in acetic acid over 5% Pd/C at 60 lb/in^2 for 3 hours at room temperature, following which recrystallization of the product gave **1.77** in 96% yield.[35] The final step of synthesizing TMIO (**1.15**) is the oxidation of 1,1,3,3-tetramethylisoindoline **1.77**. This oxidation has been achieved by two different methods. Griffiths and co-workers have achieved this oxidation by reacting **1.77** with 30% H_2O_2, $Na_2WO_4.2H_2O$ and $NaHCO_3$ in methanol. Recrystallization of the product gave the target **1.15** with a yield of 92%.[35] An alternative approach for ring substituted 1,1,3,3-tetramethylisoindolines (**1.93**) has been reported using *m*-chloroperbenzoic acid (*m*CPBA) in CH_2Cl_2 (Scheme 1.26). This gave the substituted nitroxide **1.93** with a yield of 97%.[109]



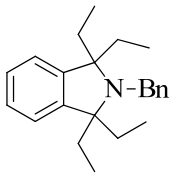
1.92 **1.93**

Scheme 1.26 Oxidation of substituted tetraalkylated isoindolines using *m*CPBA.

Caldararo and co-workers have also reported a method to perform both step (b) and (c) of Scheme 1.21 together in a one-pot reaction.[110] In this method, compound **1.76** was reacted with H_2/Pd/C in methanol and then treated to subsequent oxidation without work-up. This gave the target TMIO (**1.15**) in a yield of 71% (90% purity by gas chromatography).[110] This constitutes a lower yield compared to the Griffiths procedure.

As the Grignard reaction typically proceeds with 28-40% yield for both 2-benzyl-1,1,3,3-tetramethylisoindoline **1.76** and 2-benzyl-1,1,3,3-tetraethylisoindoline **1.94**, this reaction is clearly the yield-limiting step of the synthesis of isoindoline nitroxides. Several groups have attempted to improve the yield of this step. In 2008, Scammells and co-workers reported a microwave synthesis of tetramethyl adduct **1.76** and tetraethyl adduct **1.94** starting from **1.75** with much improved yields.[111] The reasons for utilizing microwave radiation for this reaction are owing to its ability to produce dramatically increased reaction rates, cleaner reaction profiles,[112] and in facilitating formation of Grignard reagents.[113-115] Although microwave radiation is excellent for preparing Grignard reagents, it is not used widely for large scale reactions, as reaction simplicity is usually an objective in this context. The Scammells study, however, did improve the yield of the Grignard step through modified conditions under microwave radiation to give an isolated yield of 60% for tetraethyl adduct **1.94** and tetramethyl adduct **1.76** for 45% yield.[111] The explanation given for the enhanced yield when using microwave radiation is the potential to

directly heat the key intermediate ions that lead to the final product. Although this microwave radiation improved the yield of the Grignard tetraalkylations, there were some significant practical limitations with regards to the scale of the reaction as well as potential risks of over-pressurisations and explosions.

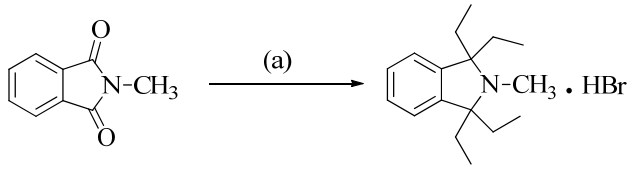


1.94

Figure 1.11 Structure of *N*-benzyl-1,1,3,3-tetraethylisoindoline **1.94**

Usually the Grignard reagent is prepared in diethylether by reacting pre-dried magnesium turnings and haloalkanes. Ethyl ethers are extremely flammable and readily form peroxides which in turn, carry the risk of explosion. After preparing Grignard reagent, excess ethyl ether is distilled off before adding phthalimide **1.75** in toluene. Removal of the ether is important, as in its presence, complete alkylation will not occur and several intermediate products mostly containing hydroxyl groups are then produced.[110] Due to these concerns, Caldararo and co-workers performed Grignard reaction in different aprotic solvents. They first used THF to prepare the Grignard reagent, but it surprisingly gave poor yields for the tetraalkylation of *N*-benzylphthalimide **1.75**.[110] The reason was stated to be the complexation of magnesium (a metal with a high coordination number) with THF, which posseses more sterically accessible and dative oxygen lone pairs than diethylether.[116] Therefore, they explored methyl-*tert*-butylether as the solvent to prepare the Grignard reagent. In this approach, they first prepared compound **1.76** by reacting **1.84** and benzylamine in refluxing toluene for 4 hours; once this reaction was completed, the reaction mixture was utilized for the Grignard without any further purification. The Grignard was prepared in methyl-*tert*-butylether to give *N*-benzyl-

1,1,3,3-tetraethylisoindoline **1.94** in a yield of 41%,[110] which was a slight improvement compared to the Griffiths' method. However, these researchers noted that this product was pure enough to utilize in the next steps without any further purification. Therefore, this simplification avoids the use of chromatography, which often leads to a substantial loss of product.[110]



1.78 **1.83**

Scheme 1.27 Formation of the HBr salt of 2-methyl-1,1,3,3-tetraethylisoindoline (**1.83**) starting from **1.78**.[103]

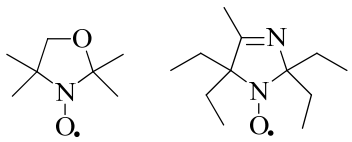
Reagents and conditions: (a) EtMgBr (excess), toluene, 110 °C, 2 h, work-up by quenching the reaction mixture (H_2O/H_2SO_4, CH_2Cl_2 extraction), 64%.

The synthesis of 2-benzyl-1,1,3,3-tetraalkylisoindoline was also attempted with slightly modified starting material **1.78**. However, the yield of the product of this reaction, 1,1,2,3,3-pentamethylisoindoline **1.79**, did not exceed 15%, although there had been previous reports of yields of 30%.[105] In addition, this pentamethylated product was contaminated with 1-ethyl-1,2,3,3-tetramethylisoindoline **1.80** (Figure 1.10), which could not be totally removed by standard purification procedures (recrystallization/ column chromatography) nor by varying the reaction conditions. Deprotection and oxidation of this mixture gave two nitroxides, TMIO (**1.15**) and 1-ethyl-1,3,3-trimethylisoindolin-2-yloxyl **1.81**, which could not be separated by recrystallization.[35] On the other hand, when utilizing **1.78** as the starting material at higher temperatures (microwave conditions), the starting material led to some complicated ring opening reactions[111] and the formation of unwanted products that contaminate the target.[35] In another attempt, substrate **1.78** was treated with excess

EtMgBr in refluxing toluene for 2 hours (Scheme 1.27). This reaction produced the HBr salt of 1,1,3,3-tetraethyl-2-methylisoindoline (**1.83**) with a yield of 64% *via* a different work-up, which involved quenching the reaction mixture with H_2O/H_2SO_4 and extracting with CH_2Cl_2.[105] Later, they converted this salt into 1,1,3,3-tetraethyl-2-methylisoindoline by stirring **1.83** in cold aqueous NaOH; however, the yield of this conversion was not reported. Of all these published procedures to date, the highest preparative-scale yield obtained for the synthesis of pure 2-benzyl-1,1,3,3-tetraalkylisoindoline is 37-41%.

1.6 HETEROCYCLE- ANNULATED NITROXIDES

Heterocyclic nitroxides are cyclic nitroxide systems that have atoms of at least two different elements as members of the nitroxide skeleton. Two examples of this nitroxide type are shown in Figure 1.12. The chemistry and applications of these nitroxides have not yet been explored widely. However, some of their important applications are listed below.



1.95 **1.96**

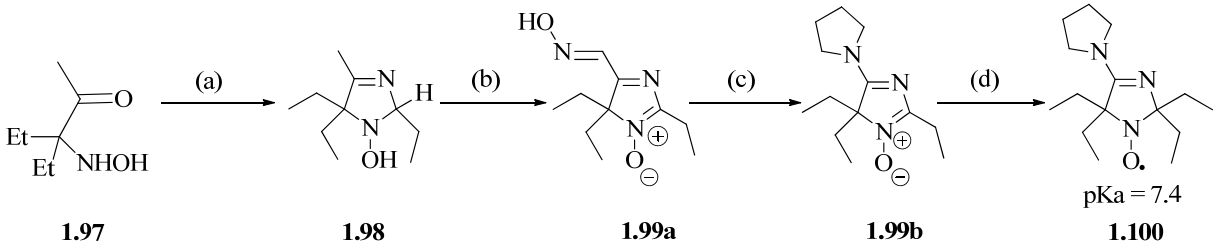
Figure 1.12 Cyclic nitroxides with heteroatoms.[117]

1.6.1 Applications of heterocyclic nitroxides

1.6.1.1 pH sensitive spin probes

Cellular pH is considered as one of the most important parameters in the biochemistry of living systems. To assess the pH changes in biological systems, stable nitroxides can be used. Imidazoline and imidazolidine-based stable

nitroxides[118a] are excellent candidates in assessing pH changes in living systems, owing to the effect of pH changes on EPR spectra.[118b]



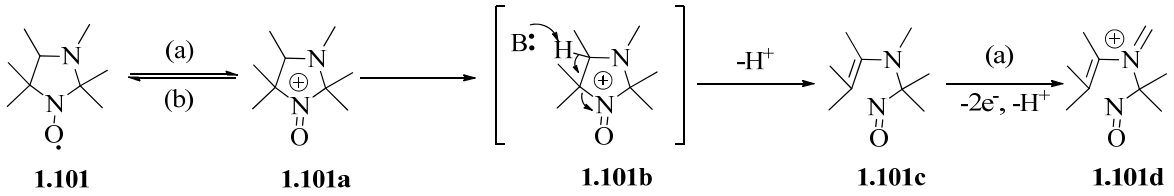
Scheme 1.28 Synthesis of a pH-sensitive nitroxide.[118b]

Reagents: (a) CH_3CH_2CHO, Et_3N (b) *i*-PrONO, Et_3N (c) 1. TsCl, Et_3N 2. Pyrrolidine (d) 1. EtMgBr 2. MnO_2[118b]

1.6.1.2 EPR probes

Imidazolidine nitroxide act as useful paramagnetic/EPR probes with high sensitivity to oxidative processes in biological systems. Usually potassium ferricyanide is utilised for the oxidation of sterically hindered hydroxylamines to nitroxides. But in the presence of imidazolidine nitroxides with potassium ferricyanide, the oxidation reaction follows a different pathway, leading to fragmentation of the nitroxide with loss of the EPR signal. This fragmentation occurs due to the abstraction of a proton from the ring of the oxoammonium cation, arising from one-electron oxidation of imidazolidine nitroxide.[119]

Increasing the steric bulk around the nitroxide moiety decreases the ascorbate-induced reduction rate, but doesn't affect the potassium ferricyanaide-induced oxidation rate. This specific sensitivity of imidazolidine nitroxides towards oxidation reactions makes them ideal candidates for the detection of peroxide radicals at high concentration of ascorbate. This observation assists in the design of imidazolidine nitroxides as paramagnetic/EPR probes with enhanced sensitivity to oxidative processes in biological systems.[119]



1.101 **1.101a** **1.101b** **1.101c** **1.101d**

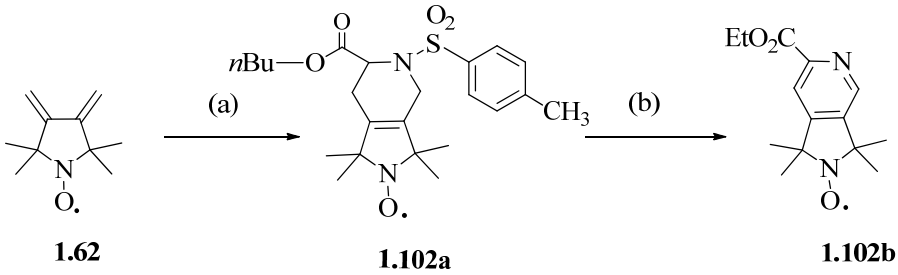
Scheme 1.29 Sequence of steps in the oxidation of imidazolidine nitroxide (**1.101**) by potassium ferricyanide.[119]

Reagents: (a) $[Fe(CN)_6]^{3-}$ (b) $[Fe(CN)_6]^{4-}$ [119]

Apart from these main applications, utilising heterocyclic nitroxides as contrast enhancing agents[8] in magnetic resonance imaging and molecular units[120a,120b] for constructing molecular magnetic materials is also important. Some structural features associated with monocyclic nitroxides containing heteroatoms (Figure 1.12, Scheme 1.28 and Scheme 1.29) give rise to disadvantages. Oxazolidine derivatives such as **1.95** are not easily oxidized, due to the electron-withdrawing effect of the heteroatom (oxygen) in the ring which destabilizes the positive charge of the resulting oxo-ammonium cation.[117] In addition, the problem of ring-opening in monocyclic nitroxides (Scheme 1.29) is also a major disadvantage.[86,119] However, these two disadvantages can be addressed if a heteroaromatic ring like pyridine can be fused to the monocyclic nitroxide. This pyridine ring fusion imparts some advantages to the nitroxide skeleton, such as rigidity towards ring-opening reactions,[86] thermal and chemical stability,[87] good sigma donor capabilities as a monodentate ligand,[121,122] and resistance towards the alteration of functions of biomolecules[123] when applied as spin labels. Owing to all these applications and advantages, the synthesis of pyridine annulated nitroxides is of considerable interest to many chemists globally.

1.6.2 Synthetic approaches to pyridine annulated nitroxides

To date, there have been three approaches described for the synthesis of pyridine-annulated nitroxides. Hideg and co-workers have introduced a synthesis of a pyridine-annulated nitroxide *via* a Diels-Alder reaction similar to the isoindoline synthesis (as shown in Scheme 1.17) starting from the paramagnetic symmetric pyrrolidine diene **1.62**.[123] In this approach, compound **1.62** was reacted with *N*-(butoxycarbonylmethylene)-*p*-toluenesulfonamide to give intermediate **1.102a**, which was then hydrolysed with KOH in EtOH followed by esterification and HNO_2 treatment, to give paramagnetic derivative **1.102b** as the final product.[123]

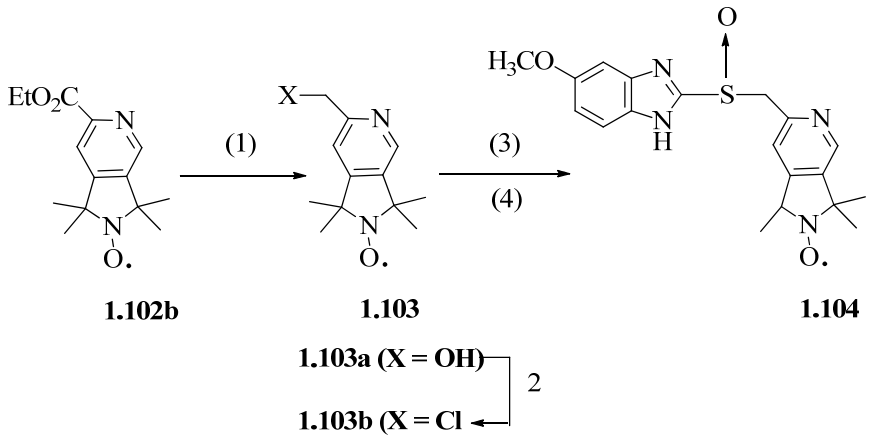


Scheme 1.30 Pyridine-annulated nitroxide synthesis by Diels-Alder reaction.[123]

Reagents and conditions: (a) *N*-(butoxycarbonylmethylene)-*p*-toluenesulfonamide (1.2 equiv)/toluene, 110 °C, 10 h (67%); (b) KOH (11.5 equiv)/EtOH, 78 °C, 3 h, then EtOH/HCl, 78 °C, 1 h, then rt, 12 h, then evaporation of volatiles, $NaNO_2$ (3.2 equiv), rt, 10 min, 73% [123]

When the synthetic yield of compound **1.62** was considered from commercially available starting material **1.67** over 6 steps (Scheme 1.17), it was 10%.[89,93] The overall synthetic yield of target nitroxide **1.102b** was 5% starting from commercially available **1.67**, which is fairly inefficient outcome for a long, multi-step synthetic sequence. The nitroxide **1.102b** was converted into several different nitroxide derivatives with lower yields by reducing ester functionality of **1.102b** to alcohol **1.103a** (9%) followed by chlorination (**1.103b**, 8%**)** with $SOCl_2$. The S-alkylation of 5-methoxy-2-mercaptobenzimidazole with paramagnetic **1.103b** followed by

oxidation with *m*CPBA, gave sulfoxide **1.104** (0.7%), which can be regarded as a paramagnetic derivative of omeprazole (Scheme 1.31).[123]

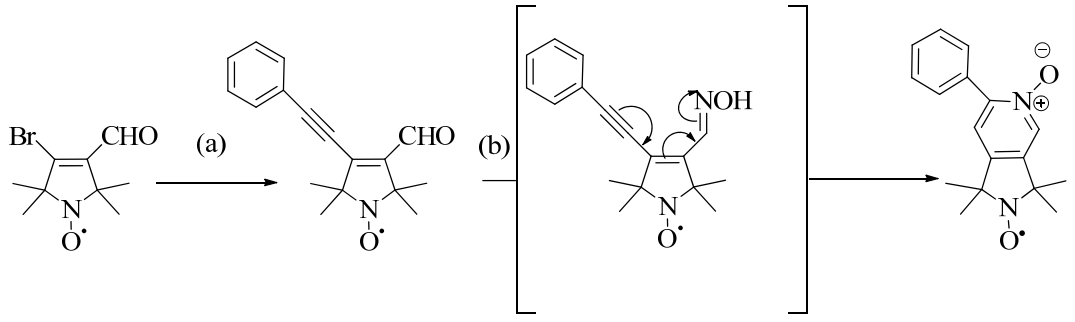


Scheme 1.31 Synthesis of pyridine annulated nitroxide derivatives from **1.102b**.[123]

Reagents and conditions: (1) $NaBH_4$ (2.5 equiv)/ EtOH, rt, 30 mins., 90%; (2) $SOCl_2$ (1.5 equiv)/ CH_2Cl_2, rt, 30 mins., 30-90%; (3) **1.103b**·HCl/ 5-methoxy-2-mercaptobenzimidazole/ NaOH (2.1 equiv)/ EtOH/H_2O, 78 °C, 2 h, 18%; (4) *m*CPBA (2 equiv)/ CH_2Cl_2, 0 °C, 20 mins., 48%.[123]

In another approach, Sar and co-workers have achieved the synthesis of a novel pyridine-fused paramagnetic nitroxide, **1.105** (Scheme 1.32), by utilising bromide **1.68** as a substrate for Sonogashira coupling with phenylacetylene in DMF in the presence of triethylamine, CuI (10 mol%), and dichlorobis(triphenylphosphine)palladium(II) as catalyst (5 mol%). The reaction afforded aldehyde **1.105a**, which was then reacted with hydroxylamine hydrochloride and K_2CO_3 in refluxing EtOH/H_2O to give target nitroxide **1.105** *via* the spontaneous cyclisation of the oxime **1.105b**.[124a]

The synthesis of compound **1.68** starting from commercially available TEMPONE (**1.19**) was achieved (Scheme 1.20) in a yield of 18%.[101,102] When the synthetic yield of **1.105** was considered from compound **1.19**, the overall yield was only 10%.

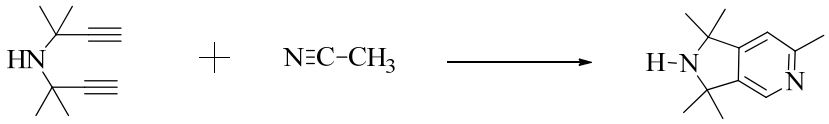


1.68 **1.105a** **1.105b** **1.105**

Scheme 1.32 Pyridine-annulated nitroxide synthesis via Sonogashira coupling.[124a]

Reagents and conditions: (a) PhC≡CH (1.0 equiv), $PdCl_2(PPh_3)_2$ (0.05 equiv), CuI (0.1 equiv), Et_3N/ DMF, 80 °C, 3 h, 72%; (b) $HONH_2$:HCl (1.0 equiv), K_2CO_3 (1.0 equiv), EtOH/ H_2O, 78 °C, 1 h, then K_2CO_3 (1.0 equiv), EtOH, 78 °C, 3 h, 82%.[124a]

A third synthetic approach to the heterocyclic nitroxide target is again based on cycloaddition reactions. In this case dipropargylamine **1.71** was reacted with acetonitrile in the presence of $CoCl_2$ and Mn powder at 80 °C under N_2 for 6 hours. Hydrolyzing the reaction mixture followed by acid-base extraction gave pure **1.106** as a solid with gas-liquid chromatographic purity determined to be 98%.[124b] However, these workers did not report a synthetic procedure for the conversion of **1.106** to the corresponding nitroxide nor its actual isolated yield.



1.71 **1.106**

Scheme 1.33 A new synthetic approach to pyridine-annulated nitroxides by cycloaddition reactions.[124b]

Reagents and conditions: $CoCl_2$, Mn powder in CH_3CN, N_2, 80 °C, 6 h.

Comparing the isolated yields obtained by the two synthetic approaches: a 10% yield by Diels-Alder reaction (Scheme 1.30, 8 steps); and a 10% yield by Sonogashira coupling (Scheme 1.32, 6 steps); and considering the advantages and applications of

this class of nitroxides, the development of a short and convenient approach for the synthesis of pyridine-fused nitroxides with an improved yield is essential.

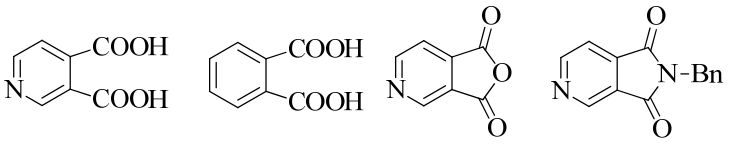
1.7 ABOUT THE PROJECT

There were two major goals of this PhD project. One of them was to improve the yields of the synthesis of both ethyl and methyl versions of *N*-benzyl-1,1,3,3-tetraalkylatedisoindoline. According to the current accepted mechanism for the tetraalkylation of *N*-benzylphthalimide **1.75** outlined in Scheme 1.25, the formation of tetraalkylated product occurs in a step-wise manner. Braslau and co-workers showed this by reacting *N*-benzylphthalimide **1.75** with 2 equivalents of MeMgBr followed by 4 equivalents of PhMgBr.[108] The formation of *meso* product **1.86b** suggested that the addition of a second equivalent of MeMgBr occurs at the face opposite to the large alkoxy magnesium salt intermediate (**1.87**). Once intermediate **1.88** is formed, the addition of a third and fourth nucleophile is much more difficult due to the poor leaving group qualities of OMgBr.[108] As a result, intermediates **1.89** and **1.91** will be formed at a much slower rate, leading to a limited yield of the final target product, **1.86b**.

Although Griffiths and co-workers have reported a versatile method of synthesizing 1,1,3,3-tetraalkylatedisoindolin-2-yloxyls over four steps, the tetraalkylation of **1.75** using a Grignard reagent is still the yield-limiting step.[35] If the starting material phthalimide **1.75** can be modified by introducing a better leaving group, the final alkylation steps may occur with much faster rates, delivering the final tetraalkylated product with an improved yield. Previously, the only structural change made to the starting material is the use of *N*-methyl version **1.78**, rather than *N*-benzyl version. However, when utilizing **1.78** as the starting material, the higher temperature led to complex ring opening reactions with microwave radiation[111] and the formation of

unwanted products that contaminated the target compound.[35] Of note, if the starting material is modified by the introduction of a good leaving group such as triflate or mesylate, the modified structure may therefore become unstable due to a higher reactivity of the leaving group. To avoid this, it is better to modify the starting material with somewhat less reactive groups such as methoxy, ethoxy *etc*. With this in mind, a new approach to the Grignard reaction has been carried out using a step-wise addition sequence. However, it is speculated that the formation of tetraalkylated isoindolines starting from the modified starting material may take place through a different intermediate more readily, delivering the final target compound in a higher yield.

The second goal of this project was to synthesise pyridine-annulated heterocyclic nitroxides *via* a short, novel approach with an improved yield. Due to the poor yields of the reaction procedures shown in Schemes 1.30, 1.31 and 1.32 and the drawbacks associated with the method shown in scheme 1.33, new approaches for the synthesis of pyridine-fused nitroxides were investigated. During this investigation, the commercial availability of a pyridine annulated dicarboxylic acid **1.106** (Figure 1.13), which is structurally closer to the starting material (1,2-benzenedicarboxylic acid **1.107**, Figure 1.13) of phthalic anhydride **1.84**, was noted. Further investigations showed that pyridine-3,4-dicarboxylic acid **1.106** had been previously converted to the corresponding anhydride **1.108** (Figure 1.13) in a good yield. This prompted us to develop a new synthetic route to pyridine-fused nitroxides starting from commercially available **1.106**, in a manner similar to the synthesis of TMIO (**1.15**) and TEIO (**1.13**). Interestingly, this method would generate non-substituted pyridine-fused pyrrolidine nitroxides, which have not been synthesized to date.



1.106 **1.107** **1.108** **1.109**

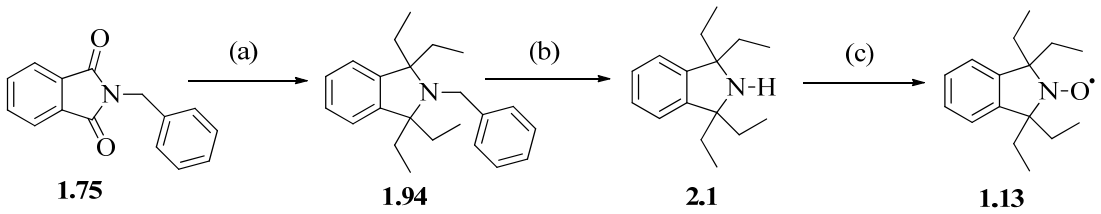
Figure 1.13 Compounds involve in synthesizing pyridine annulated nitroxides.

Since starting material **1.109** (Figure 1.13) and *N*-benzylphthalimide **1.75** are structurally similar (with the exception of the N atom in the aromatic ring), and the proposed synthetic steps to tetraalkylated pyridine nitroxides are similar to the synthesis of isoindoline nitroxides (TMIO and TEIO), insights into the relevant mechanistic steps are also expected to be somewhat similar to those of the isoindoline system. Therefore, alkylation of imide **1.109** *via* a Grignard approach is speculated to be yield-limiting, and the cause of the poor yield may be predictable by comparing the results with that of the isoindoline systems. Improving the yields of these pyridine-annulated nitroxides will be attempted in a similar way to that explained above by modifying imide **1.109** through introducing a good leaving group, such as triflate, mesylate *etc*., or a less activated leaving group such as methoxy, ethoxy, *etc*.

Chapter 2: IMPROVING THE YIELD OF THE GRIGNARD TETRAETHYLATION OF *N*-BENZYLPHTHALIMIDE

2.1 BACKGROUND

Grignard tetraethylation of *N*-benzylphthalimide **1.75** is the most critical step in the synthesis of 1,1,3,3-tetraethylisoindolin-2-yloxyl **1.13** (commonly abbreviated as TEIO). Owing to the poor yield of tetraethyl adduct **1.94** generated by this reaction, it is recognised as the yield-limiting step in the synthesis of TEIO (**1.13**). This step has been carried out by treating **1.75** with excess ethylmagnesium halide in refluxing toluene (for 3-5 hours), whereby and the maximum yield obtained for tetraethyl adduct **1.94** was 28-40%.[111]



1.75 **1.94** **2.1** **1.13**

Scheme 2.1 Synthesis of TEIO **1.13** via Grignard addition to *N*-benzylphthalimide[88]

Reagents and conditions: (a) EtBr (8.0 equiv), Mg (8.5 equiv), MeOtBr, N_2, rt, then Toluene reflux, 4 h, **15** (1.0 equiv), 40%; (b) **1.94** AcOH, H_2/Pd/C, rt, 3 h, 60 lb/in^2, NaOH (10%), Et_2O, 95%; (c) **2.1**, MeOH/CH_3CN, then $NaHCO_3$ (aq., 1.0 equiv), $(NH_4)_6Mo_7O_{24}.4H_2O$ (0.009 equiv), 45 °C, then H_2O_2 (30%, 4.0 equiv), 2 h, 88%.[88]

The synthesis of TEIO (**1.13**) with an improved yield has become a goal of wide-spread interest due to a wide variety of applications and advantages possessed by the resultant nitroxide. Apart from the previously mentioned advantages of isoindoline nitroxides such as structural rigidity towards ring opening reactions,[86] chemical and thermal stability[87] and characteristic narrow EPR linewidths,[87] resistance towards

bioreduction in biological systems due to the bulky ethyl group has made TEIO the most persistent nitroxide radical in the presence of reducing agents.[125] Some of the well-known applications of TEIO such as successful initiators in nitroxide-mediated polymerization[45,55,126,127] and versatile free radical trapping agents[39] are also of notable importance.

Previous approaches to the tetraethylation of phthalimide **1.75** under different experimental conditions as well as with different ethyl halides and aprotic solvents have been explored in order to improve the yield of this step. Two approaches are most notable in terms of improving the yield. Caldararo and co-workers patented a slightly higher yielding procedure for the synthesis of tetraethyl adduct **1.94** using methyl-*tert*-butylether[110] instead of diethylether as the solvent. Preparing EtMgBr in methyl-*tert*-butylether avoided unwanted peroxide formation in the reaction mixture which could lead to explosions and the formation of insoluble impurities. This method records the highest preparative scale yield (41%)[110] for the synthesis of tetraethyl product to date. Secondly, Scammells *et al.* described the applications of microwave assisted Grignard reactions for the synthesis of tetraethyl adduct **1.94** with a yield of 60%.[111] Although the reaction gave significantly higher yield, explosion risk and the difficulties in using Microwave for large scale preparations have limited the wide-spread use of this method.[111]

Although Grignard reactions represent one of the most powerful tools in synthetic organic chemistry, surprisingly little understanding of their mechanisms and the behaviour of Grignard reagents in solutions is known. The mechanism of forming tetraethyl adduct **1.94** *via* Grignard tetraethylation of phthalimide **1.75** has been investigated by reacting phthalimide **1.75** with 2 equivalents of MeMgBr followed by 4 equivalents of PhMgBr.[108] Formation of *meso* 1,3-dimethyl-1,3-diphenyl-2-

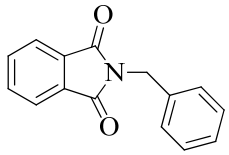
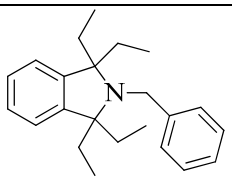
benzylisoindoline **1.86b** suggested that the addition of the second equivalent of MeMgBr occurs at the face opposite to the large alkoxymagnesium salt OMgBr, giving intermediate **1.88** (Scheme 1.25, Chapter 1). Addition of third and fourth equivalents of PhMgBr to **1.88** occurs with similar steric considerations as the addition of the MeMgBr occurred, giving the final product **1.86b** in 25% yield.[108] However, it is believed that third and fourth alkylation are relatively difficult owing to the poor leaving group qualities of alkoxymagnesium salts, as well as the steric hindrance created by the first and second alkyl groups, resulting in low yields for the final tetraalkylated product.[108]

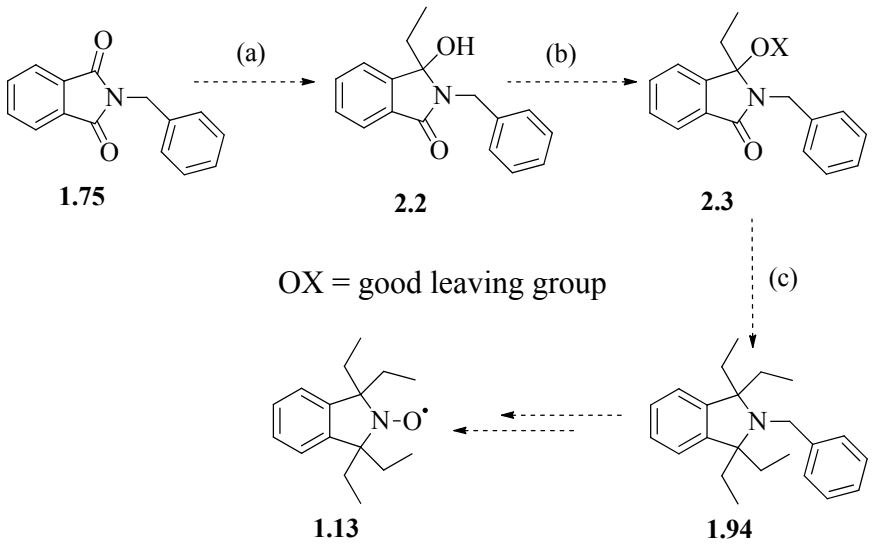
A slightly different method has been explored starting from *N*-methylphthalimide **1.78**;[105] however this gives the HBr salt of 1,1,3,3-tetraethyl-2-methylisoindoline (**1.93**, Scheme 1.27) with a good yield, also incorporating a different work-up. The workers involved did not however report the procedure or any attempts to convert this salt into the corresponding nitroxide. In another experiment, tetraethyl adduct **1.94** was synthesised *via* a slightly modified procedure[128] from that patented by Caldararo. According to this method, Grignard reagent EtMgBr was prepared by refluxing magnesium powder and ethylbromide in Et_2O for 3 hours and the excess ether then removed under high vacuum conditions. After adding phthalimide **1.75** in toluene to the resulting EtMgBr, the reaction mixture was refluxed for 18 hours, which generated 40% yield for tetraethyl adduct **1.94**.[128] Of all these published procedures, the highest practical preparative scale yield obtained for **1.94** remains modest (41%). This thesis describes a step-wise addition sequence for the tetraethylation of phthalimide **1.75**, which delivers improved isolated yields up to 50% over two steps.

2.2 SPECIFIC OUTLINE OF THE PROJECT

The main objective of this project was improving the yield of the target 1,1,3,3-tetraethyl-2-benzylisoindoline **1.94** by introducing a better leaving group to the substrate *N*-benzylphthalimide **1.75**. If the yield of the target molecule **1.94** could be increased by this method, the final nitroxide TEIO (**1.13**) would be delivered with a higher yield.

Table 2.1 Structures of the substrate and the synthetic target of the project

Substrate	Target
 **1.75**	 **1.94**



Scheme 2.2 Proposed reaction pathway to improve the yield of **1.94**

Reagents and conditions of steps (a), (b) and (c) will be discussed in detail in the following section.

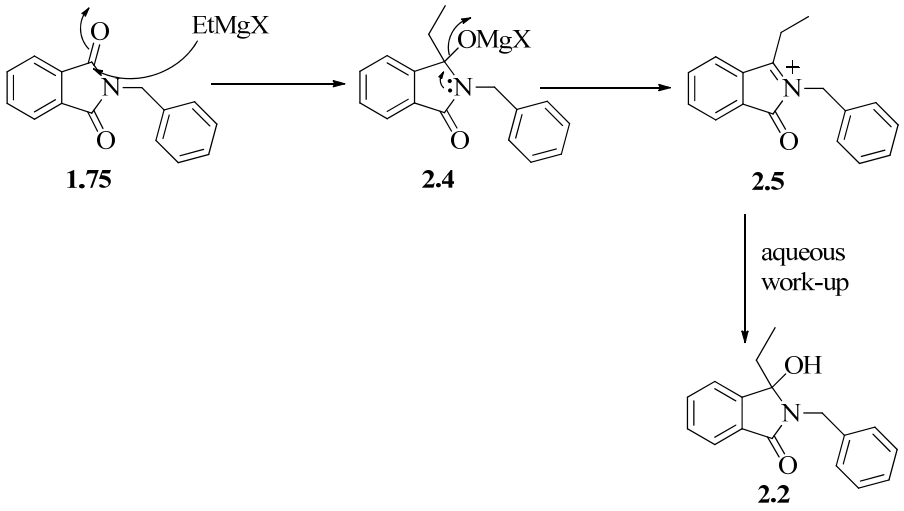
2.3 RESULTS AND DISCUSSION

This section will describe the reactions, experimental conditions, reagents used and the observations/challengers encountered during the synthesis of **1.94** starting from **1.75**. *N*-benzylphthalimide **1.75** was chosen as the starting material throughout the studies over *N*-methylphthalimide **1.78**, as phthalimide **1.75** showed resistance for ring opening reactions even at extremely high temperature generated under microwave conditions. *N*-benzylphthalimide **1.75** was synthesized according to literature, which involved *N*-benzylation of phthalic anhydride **1.84** in refluxing acetic acid, resulting in a yield of 98%.[107] The mechanism which explains this reaction is often referred to as amino-de-acyloxy-substitution, shown in Scheme 1.22 in Chapter 1.

2.3.1 Synthesis of 2-benzyl-3-ethyl-3-hydroxyisoindolin-1-one **2.2**

2.3.1.1 Background

The ethylhydroxyamide **2.2** was first synthesized by two German scientists in 1965 by treating *N*-benzylphthalimide **1.75** with 2-3 equivalents of ethylmagnesium halide in toluene at 30-50 °C.[129] However, the complete experimental procedure for synthesizing **2.2** and the characterization of this unusual compound has not been published previously. The formation of ethylhydroxy amide **2.2** is likely to proceed by monoaddition of EtMgX to one of the carbonyl groups of the imide, followed by conversion to the alcohol during the aqueous work-up.



1.75 **2.4** **2.5** **2.2**

Scheme 2.3 Proposed mechanism for generation of **2.2**[108]

2.3.1.2 Synthesis and optimization of **2.2**

A series of Grignard reactions was carried out with increasing the equivalents of EtMgI (1.0 equivalent to 2.5 equivalents) under the stated reaction time and temperature (in trial 1, Table 2.2), however, the highest isolated yield for **2.2** was obtained (46%, trial 1, Table 2.2) when 2.5 equivalents of EtMgI was used. Therefore, 2.5 equivalents of EtMgI were employed as the optimised amount to improve the yield of **2.2** as shown in Table 2.2 below. After reacting phthalimide **1.75** with EtMgI in refluxing toluene for 30 minutes under an argon atmosphere (Trial 1, Table 2.2), the reaction mixture was then diluted with hexane, and filtered through Celite. The Celite was washed with hexane and the filtrate collected was evaporated under reduced pressure. The analysis of the resultant solid crude by HPLC showed the presence of tetraethyl adduct **1.94**. The residue on the Celite was then quenched with isopropanol followed by water. The mixture was extracted with chloroform, which gave the major portion of the title compound **2.2** (46%), as identified by ^{1}H-NMR spectroscopy. The reflux time was reduced to 15 minutes in the second trial in order to limit the formation of **1.94**. This trial improved the yield of **2.2** to 51%.

Table 2.2 Summarised % reaction yields of **2.2** under different experimental conditions.

Trial	Equiv. of Mg	Equiv. of C_2H_5I	Reaction temp. (°C)	Reaction Time (min.)	% yield of **2.2**
1	2.5	2.5	110	30	46
2	2.5	2.5	110	15	51
3	2.5	2.5	rt	60	62

Since monoethylation of phthalimide **1.75** has been previously carried out at temperatures as low as 30 °C,[129] it was decided to perform this reaction at room temperature for 1 hour (trial 3, Table 2.2). Tetraethylated adduct **1.94** was not subsequently observed in the hexane extraction, but an improved yield of **2.2** (62%) was isolated from the chloroform extraction. Although monoethylation was expected to be a fast reaction, the yield of **2.2** obtained from trial 3 remained modest. Presumably some amount of product **2.2** formed in the reaction is trapped in Celite during the work-up. Therefore, the reaction mixture obtained (by reacting phthalimide **1.75** with 2.5 equivalents of EtMgI in toluene at room temperature for 1 hour) was directly quenched with aqueous saturated NH_4Cl for 3-5 hours with stirring, and the toluene layer then separated to give a small amount of the desired product **2.2**. However, the major portion of the target **2.2** was obtained by chloroform extraction of an aqueous NH_4Cl layer. Combining the two crude products of **2.2** gave 98% total crude yield and 86% recrystallised yield with 98% purity (by high pressure liquid chromatography, HPLC). The ^{1}H-NMR spectrum of the product confirmed the structural assignment with characteristic two doublets for the benzylic methylene protons, which is typical for monoalkylated derivatives of phthalimide **1.75**.

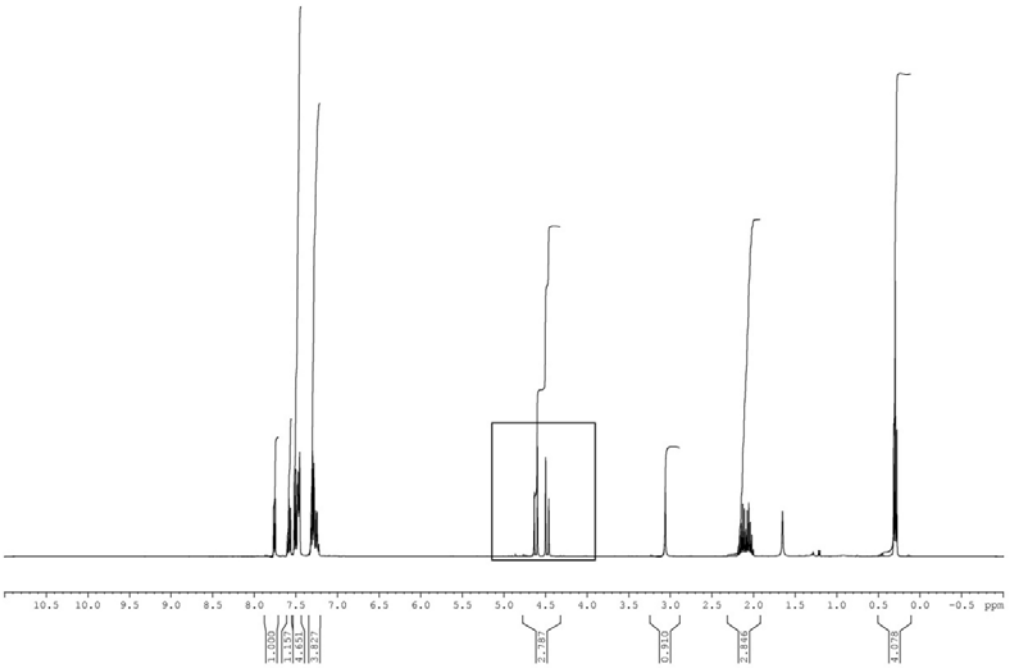


Figure 2.1 Two doublets given for benzylic methylene protons in the ^{1}H-NMR spectrum of ethylhydroxy amide **2.2**

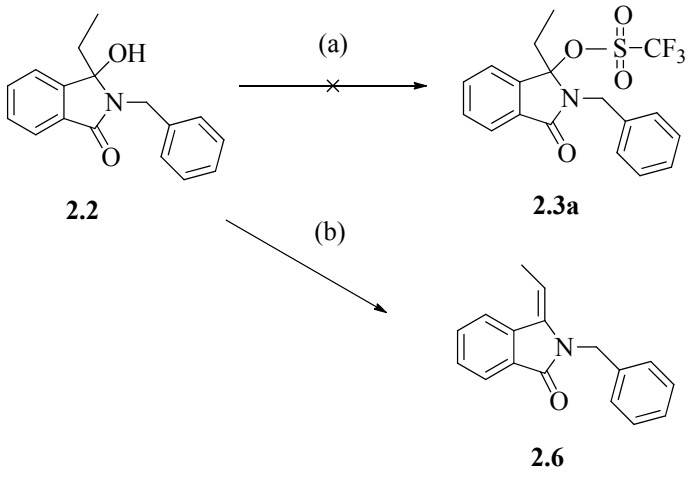
2.3.2 Attempts to improve the yield of tetraethyl adduct **1.94** by converting ethylhydroxy **2.2** into a good leaving group

2.3.2.1 Background

Reagents such as *para*-toluenesulfonyl chloride, trifluoromethanesulfonyl chloride, methanesulfonyl chloride, trimethylsilyl chloride *etc*. are often reacted with alcohols in the presence of bases such as pyridine or triethyl amine in order to convert the alcohol C-O bonds into good leaving groups. It was hypothesized that by reacting with a suitable nucleophile, the leaving group character would be enhanced to give the target **1.94** in improved yield.[130,131] In the current study, compound **2.2** will be reacted with trifluoromethanesulfonyl chloride (triflic chloride) in the presence of a base in order to synthesize the desired triflate. This triflated product will then be employed as the substrate for the tetraethylation reaction.

2.3.2.2 Attempted synthesis of **2.3**

Compound **2.2** was treated with triflic chloride in the presence of triethylamine in dichloromethane (DCM) at 0-10 °C over a period of 10 minutes under an argon atmosphere. The temperature was allowed to increase to ambient and the reaction was stirred for one hour. When the mixture was analysed by thin layer chromatography (TLC) within the first ten minutes and after another 10 minutes, a new spot was observed. The reaction mixture was washed with 2M HCl (and then brine) in order to remove triethylamine, and the component was isolated by column chromatography (hexane:ethyl acetate, 4:1) and identified by ^{1}H-NMR and mass spectrometry as being exocyclic amide **2.6**, rather than desired triflate **2.3a**.



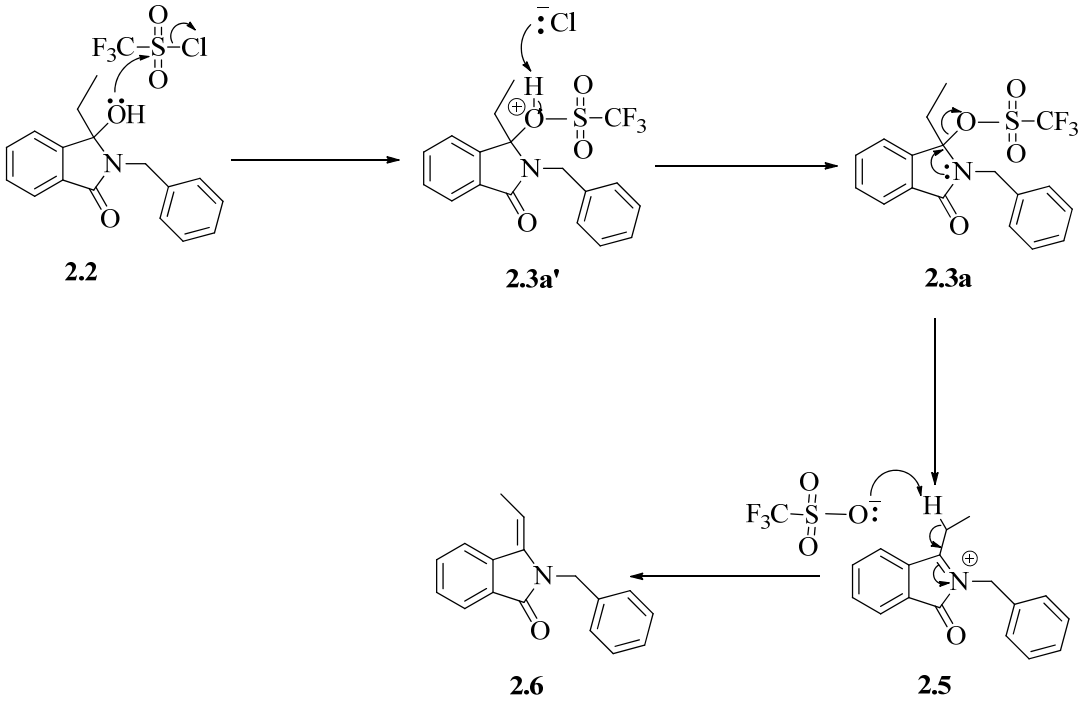
2.2 **2.3a** **2.6**

Scheme 2.4 An attempt to synthesize a triflated product

Reagents and conditions: (a) and (b) TfCl (2.0 equiv), Et_3N (2.0 equiv), DCM, argon, 0-10 °C to rt, 1 h.

In another experiment, triflic chloride was added to the starting material at -10 °C and the mixture stirred for 15 minutes at this temperature. Analysis of the reaction mixture by TLC again indicated only the formation of the exocyclic amide **2.6**. As proton abstraction by Et_3N promotes the elimination process, triflic chloride was added to substrate **2.2** in DCM at 0-10 °C in the absence of Et_3N. However, these

conditions also gave exocyclic alkene **2.6**. The proposed mechanism for the formation of alkene **2.6** is shown in Scheme 2.5 below. In all these experiments, the ^{1}H-NMR spectrum of the crude product showed two components in the ratio of 10:1 which are most likely *cis*/*trans* isomers of the alkene. However, recrystallization of the mixture from ethanol gave only the major isomer, which was later identified as *trans* product **2.6** by NOESY spectroscopy.



Scheme 2.5 Proposed mechanism to form **2.6**

Subsequently, compound **2.2** was treated with less reactive agents such as methanesulfonyl chloride, acetyl chloride and acetic anhydride in the presence of Et_3N (in the case of acetic anhydride, pyridine was used as the base), as shown in Table 2.3 below. The product of all these reactions was alkene **2.6**. Undertaking these reactions without a strong base, or at low temperature, still produced the same elimination product **2.6**. These results indicated a less reactive leaving group was necessary as a potential strategy for improving the yield of tetraethyl adduct **1.94**.

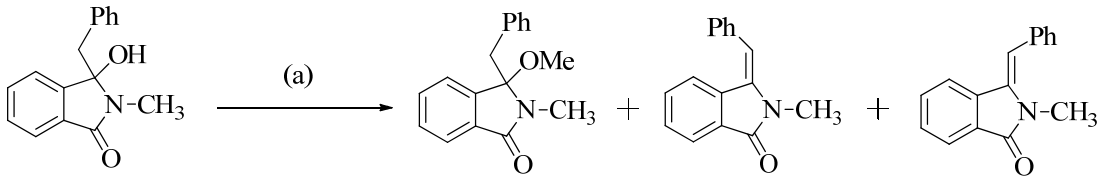
Table 2.3 Summarised experimental conditions and the % yields to form **2.6** (with Et_3N) at 0-10 °C-rt for 1 h.

Entry	Type of acid chloride used	**2.6** (%)
1	triflic chloride	78
2	mesyl chloride	89
3	acetyl chloride	67

2.3.3 Synthesis of 2-benzyl-3-ethyl-3-methoxyisoindolin-1-one **2.3b**

2.3.3.1 Background

It was hypothesized that a more effective strategy would be to modify compound **2.2** with a comparatively less effective leaving group, such as the methoxy group. In the literature, methylation reactions have been performed with hydroxyisoindolinone systems previously, but not with compound **2.2**. As an example, hydroxyisoindolinone **2.7** was refluxed with acidic methanol to produce methoxyisoindolinone **2.8a**. However, this reaction also produced two other major products, **2.8b** and **2.8c** (Scheme 2.6).[132,133]

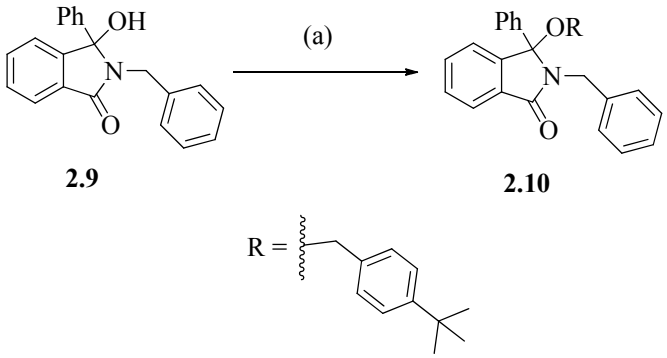


2.7 **2.8a** **2.8b** **2.8c**

Scheme 2.6 A different approach of synthesizing methoxyether containing isoindolinones[133]

Reagents and conditions: (a) MeOH/HCl [2.5 mL, 1.5M], reflux.

In another procedure (Scheme 2.7), *N*-benzylhydroxyisoindolinone **2.9** was reacted with *n*PrOH and phenol in the presence of Et_3N in tetrahydrofuran to produce **2.10**, which is another type of isoindolinone containing ether.[134]



Scheme 2.7 Another approach of synthesizing ether containing isoindolinones[134]

Reagents: (a) 1) $SOCl_2$, DMF (cat.), THF, 2) 4-*t*BuBnOH, Et_3N, THF.

Detailed experimental procedures for these reactions have not been published. Herein we report a novel method to synthesize 2-benzyl-3-ethyl-3-methoxyisoindolin-1-one **2.3b**, in an isolated yield of over 85%.

2.3.3.2 Synthetic experiments and optimization of **2.3b**

Freshly ground NaOH and Cs_2CO_3 were utilized as bases in two separate reactions along with **2.2** and MeI in dry THF. Cs_2CO_3 was used in one reaction owing to its higher basicity and solubility in organic solvents. Bases such as Et_3N and pyridine were not used due to the difficulties in removing them from the final product.

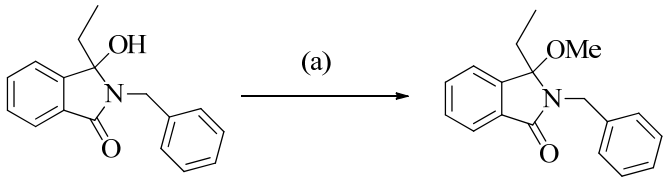
Freshly ground base was added to compound **2.2** and dissolved in THF under an argon atmosphere at room temperature. Then MeI was added to this mixture and the resulting reaction mixture was stirred at room temperature until the substrate was consumed (by TLC). After 36 hours of stirring, compound **2.2** was completely consumed in reactions employing NaOH, but some substrate was still present when Cs_2CO_3 was used as the base. The latter reaction required a further 10 hours to go to completion. NaOH was therefore chosen as the base over Cs_2CO_3 for further optimization studies. After the reaction, THF was removed under reduced pressure and the product obtained dissolved in dichloromethane. The organic phase was washed with water to remove NaOH. Evaporation of the organic layer gave 2-

benzyl-3-ethyl-3-methoxyisoindolin-1-one, which was further recrystallised from hexane.

Table 2.4 Optimization of the % yield of **2.3b** (reactions at rt).

Trial	Equivalents of NaOH	Equivalents of MeI	time (hrs)	crude **2.3b** (%)
1	1.5	1.5	48	62
2	2.0	2.0	48	71
3	3.0	3.0	36	78
4	4.0	4.0	36	93

According to the data in Table 2.3, the yield of **2.3b** was improved from 60% to 90% by increasing the equivalents of NaOH and MeI from 1.5 to 4.0. The best crude yield obtained for **2.3b** was 93% and recrystallisation of the crude by hexane gave crystals in 87% yield.



2.2 **2.3b**

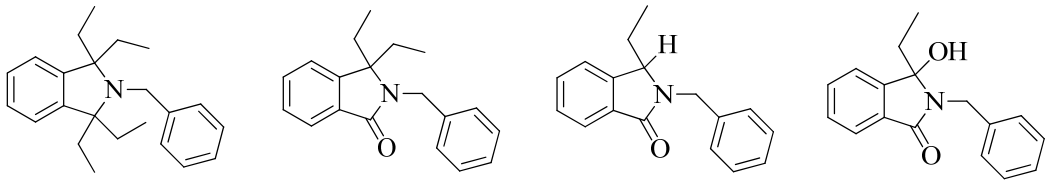
Scheme 2.8 Synthesis of methoxy ether **2.3b** from compound **2.2**

Reagents and conditions: (a) NaOH (4.0 equiv), MeI (4.0 equiv), THF, argon, rt, 36 h, 87%.

2.3.4 Synthesis of 1,1,3,3-Tetraethyl-2-benzylisoindoline **1.94** using modified starting material **2.3b**

After modifying phthalimide **1.75** with a less effective leaving group such as a methoxy group, the product was utilised as the substrate for further ethylation with the expectation that the yield of tetraethyl adduct **1.94** would be improved due to the

enhance character of the leaving group. Methoxy amide **2.3b** in dry toluene was added to 6 equivalents of EtMgI prepared in dry diethyl ether and refluxed in toluene for 3 hours. The reaction mixture was allowed to reach room temperature and quenched with saturated NH_4Cl. The toluene layer was then separated, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The oily material obtained (upon evaporation of the toluene) revealed the presence of 4 components on a TLC plate with hexane: ethyl acetate 4:1 as the eluent. The aqueous NH_4Cl layer was extracted with chloroform, dried over anhydrous Na_2SO_4 and evaporated to give a crude which contained three of the components observed in the previous product. The NH_4Cl work-up used for this Grignard reaction is more efficient than the hexane work-up, as it ensures all organic products (except basic products) formed in the reaction are partitioned into the organic phase. Therefore the NH_4Cl work-up was chosen over the hexane work-up.



1.94 **2.11** **2.12** **2.2**

Scheme 2.9 Products obtained by reacting **2.3b** with excess EtMgI

The products isolated from the evaporation of both organic layers were combined and analysed by HPLC (Figure 2.2). A short silica column was run for this isolated mixture of products with hexane: ethyl acetate 4:1, and the four prodcuts were isolated and identified by ^{1}H-NMR spectroscopy as tetraethyl adduct **1.94**, diethyl amide **2.11**, monoethyl amide **2.12** and ethylhydroxy amide **2.2** (Scheme 2.9). These four products were identified clearly as four peaks in the chromatogram (shown in Figure 2.2) by running the isolated samples by HPLC using the same conditions. The HPLC chromatogram of the reaction mixture (Figure 2.2) showed the presence of

traces of toluene and some toluene-derived impurities, apart from the four components of the reaction mixture. Toluene and the impurities derived from toluene were identified by evaporating toluene and analysing the resulting residue by HPLC. HPLC allowed the direct analysis of the reaction, so that modification of the reaction conditions could be exploited to optimise the yield of the desired tetraethyl adduct. (The relative HPLC yields of the four alkyl isoindoline products; **1.94**, **2.11**, **2.12** and **2.2**, as shown in Table 2.5, were calculated based on the area under the curve of the chromatogram, as shown in Figure 2.2 below. All the yields shown in Table 2.5 reflect the area of a particular peak compared to all the peaks in the chromatogram, so the yields represent relative HPLC peak sizes. Note: An assumption was made that all these four species have an equivalent UV absorption).

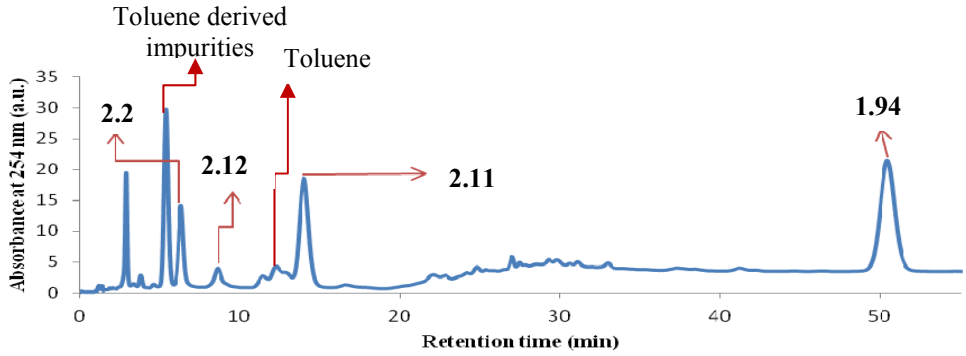


Figure 2.2 HPLC chromatogram given for the Grignard tetraethylation of **2.3b**.

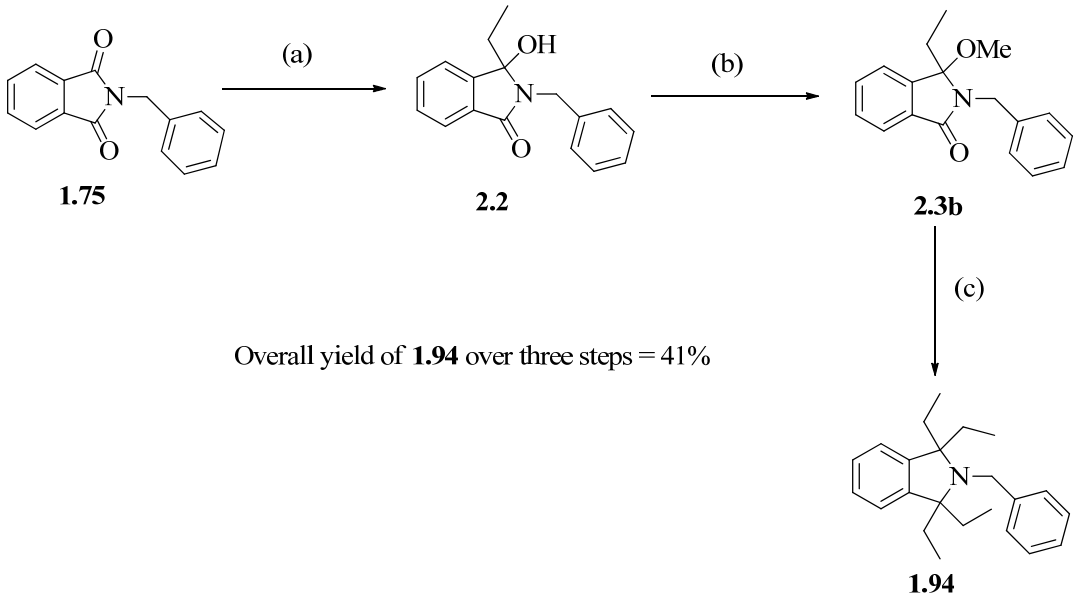
Elution method: MeOH/H_2O (65:35) for 17 minutes, then ramp to MeOH/H_2O (90:10) over 10 minutes, then held at MeOH/H_2O (90:10) for 30 minutes.

2.3.4.1 Optimizing the yield of **1.94** using **2.3b** as the substrate under different experimental conditions

According to the relative HPLC yields shown in Table 2.5 below, heating the reaction mixture at 80 °C for 14 hours (Entry 1) gave only 11% of tetraethyl adduct **1.94** in the reaction mixture, alongside a higher level of **2.2** (54%). Extending the reaction time from 14 hours to 48 hours at the same temperature (Entry 2 and 3)

showed a significant drop in the relative amount of **1.94** (22% to 5%), with an increase in the relative yield of **2.2** (69%). Therefore the temperature was increased to 110 °C and the yield of **1.94** increased accordingly. The maximum relative yield obtained for **1.94** was 60% and the relative amounts of other three products were notably small (Entry 9). The temperature of the reaction mixture was further increased to 140 °C by replacing toluene with xylene (Entry 13), and the relative amount of tetraethyl adduct **1.94** was slightly improved to 63% within 72 hours. Extending the reaction time to 120 hours at 140 °C gave similar results in Entry 13. To support the reproducibility of relative HPLC result (63%), the reaction was repeated under the same experimental conditions and a similar HPLC result (62%) was observed. An actual isolated yield of 55% was obtained by silica column chromatography in order to test the validity of this relative HPLC amount. Since the actual isolated yield (55%) was close to the relative HPLC yield (63%), the approach to improving the yield of **1.94** based on the relative HPLC peak heights/areas was validated.

Surprisingly, increasing the equivalents of EtMgI from 6 to 12 decreased the levels of **1.94** from 50% (Entry 8) to 27% (Entry 11). Such an outcome may result from a decrease in the concentration of EtMgI due to the presence of competitive Wurtz-like self-coupling reactions, which have been identified as major side-reactions occuring during the formation of organomagnesium halides.[135] Finally, the overall isolated yield of **1.94** over three steps was calculated and is shown in Scheme 2.10 below (41%). This value is still similar to the current isolated yield of **1.94** which was obtained over one step. Therefore, an alternative synthetic strategy to the use of OMe or OTf/OMs/OAc would be necessary to improve the yield of **1.94**.



Scheme 2.10 Synthetic route and the overall yield of **1.94** over 3 steps.

Reagents and conditions: (a) EtI (2.5 equiv), Mg (2.5 equiv), Et_2O, Ar, rt, 1 h with **1.75** (1.0 equiv) in toluene, 86%; (b)) NaOH (4.0 equiv), MeI (4.0 equiv), THF, **2.2** (1.0 equiv), Ar, rt, 36 h, 87%; (c) EtI (6.0 equiv), Mg (8.0 equiv), Et_2O, Ar, rt, xylene, reflux, 72 h, **2.3b** (1.0 equiv), 55%.

Initially it was speculated that **2.11** and **2.2** may act as intermediates on the pathway of forming **1.94**. Therefore, in one of the attempts to find out an alternative synthetic strategy to improve the yield of **1.94**, ethylmethoxy amide **2.3b** was reacted with 6 equivalents of EtMgI at reflux in toluene for 24 hours; after this time, another batch of 10 equivalents of EtMgI was added to this and the reflux was continued for another 72 hours. It was expected that any of the **2.11** and **2.2** (Scheme 2.9) formed in the reaction mixture after 24 hour reflux would be converted to **1.94** by the second addition of excess EtMgI. When the reaction mixture was analysed by HPLC, a relative yield of 43% (HPLC product ratio) for **1.94** was found, which was not improved as expected, and this relative yield was comparable to that obtained with 6 equivalents of EtMgI followed by 96 h reflux (Entry 8, Table 2.5). If either **2.11** or **2.2** are intermediates in the formation of **1.94**, the yield of **1.94** could be expected to be improved by starting the process at a more advanced stage. Therefore, Grignard

reactions were carried out on the three products obtained by tetraethylation of **2.3b**; that is, **2.11**, **2.12** and **2.2**, in order to investigate the theory that these compounds are intermediates in the formation of **1.94**.

Table 2.5 HPLC product ratios from the reaction of **2.3b** with EtMgI under various reaction conditions.

Entry	Reaction Temperature (°C)	Reaction time (hrs)	Equiv. EtI	Equiv. Mg	HPLC product ratio (%)			
					1.94	**2.11**	**2.12**	**2.2**
1	80	14	6.0	8.0	11	26	9	54
2	80	24	6.0	8.0	22	29	16	33
3	80	48	6.0	8.0	5	17	9	69
4	110	3	6.0	8.0	24	20	5	54
5	110	14	6.0	8.0	17	26	8	49
6	110	24	6.0	8.0	26	26	14	34
7	110	72	6.0	8.0	43	26	11	20
8	110	96	6.0	8.0	50	19	11	20
9	110	120	6.0	8.0	60	16	11	13
10	110	72	12.0	16.0	25	14	14	47
11	110	96	12.0	16.0	27	20	18	35
12	140[a]	24	6.0	8.0	51	27	9	13
13	140[a]	72	6.0	8.0	63	19	12	6

[a] Using xylenes as solvent

Equivalents of EtI and Mg in the table were relative to 1.0 equivalent of **1.75**

2.3.4.2 Improving the yield of tetraethyl adduct **1.94** using an alternative synthetic startegy

Since diethyl amide **2.11** contains two ethyl groups on the same side, one equivalent of **2.11** was treated with a slightly decreased amount of Grignard reagent (4.0 equivalents of EtMgI) as compared to reaction starting from **1.75** at 110 °C for 96 hours. The reaction mixture was then quenched with saturated aqueous NH_4Cl and the products obtained from both the toluene layer and the aqueous NH_4Cl layer were added together and analysed by HPLC (Figure 2.3). Surprisingly, only a small amount of **2.11** was converted to **1.94**; most of the starting material remained unreacted (75 mg scale, Entry 1, Table 2.6). To check the reproducibility of the result, this reaction was repeated under the same experimental conditions (Entry 2, Table 2.6) and a similar result was observed by HPLC. It is suggested that correct approach to the remaining amide carbonyl group (of **2.11**) by EtMgI reagent during the reaction may be hindered by the steric crowding imposed by the two ethyl groups across the ring.

Monoethyl amide **2.12** was reacted with 6 equivalents of EtMgI in refluxing toluene for 72 hours. After quenching the reaction mixture with saturated NH_4Cl, both toluene layer and the chloroform extraction of the aqueous NH_4Cl layer were analysed by HPLC. No substrate **2.12** could be detected, but equally there was no tetraethyl adduct **1.94** or ethylhydroxy amide **2.2** in the reaction mixture. Analysis of the complex reaction mixture by ^{1}H-NMR spectroscopy showed the absence of any typical alkyl isoindoline signals (peaks related to four ethyl groups of the tetraethyl adduct **1.94**). Further analysis of the reaction outcome was not continued, as none of the target could be detected in the chromatogram. It was concluded from these results that both **2.11** and **2.12** are not precursors for the final product and may therefore

represent "dead-end" side reactions that limit the yield when **2.3b** is used as the substrate.

Table 2.6 HPLC product ratios obtained from the Grignard ethylation of **2.11** and **2.2** as the starting material.

Entry	Reaction Temperature (°C)	Reaction time (hrs)	Equiv. EtI	Equiv. Mg	HPLC product ratio (%) **1.94**	**2.11**	**2.12**	**2.2**
1[a]	110	96	4.0	6.0	3	96	-	-
2[a]	110	96	4.0	6.0	3	97	-	-
3[b]	110	72	6.0	8.0	80	17	-	3
4[b]	110	72	6.0	8.0	78	17	-	5
5[b]*	110	72	6.0	8.0	80	9	-	11

[a] diethylamide **2.11** as the starting material, [b] ethylhydroxyamide **2.2** as the starting material, *8.5 g scale reaction; Equivalents of EtI and Mg were relative to 1 equivalent of **2.11** and 1 equivalent of **2.2**

Another approach investigated was to use the mono-ethylhydroxyamide **2.2** as the substrate for Grignard alkylation. When the reaction mixture was quenched with saturated NH_4Cl and analysed by HPLC, most of the starting material had been consumed and the desired target (**1.94**) comprised 80% of the products detected (Entry 3, Table 2.6). On the other hand, product **2.12** was not observed in the HPLC and only a low level of **2.11** (17%) was detected. The reproducibility of the relative product ratios of **1.94** in Entry 3 was determined by re-running this experiment in duplicate on 100 mg scale (Entry 4, Table 2.6) and 8.50 g scale (Entry 5, Table 2.6). The relevance of the HPLC analysis to the actual product yields was determined by isolating the product *via* column chromatography. An actual isolated yield of 70% was determined for 100 mg scale reaction. When the reaction was scaled up to 8.5 g of the ethylhydroxy amide **2.2**, the isolated yield of tetraethyl adduct **1.94** was 57%.

This was an improvement upon the best yield recorded in the literature. The observed difference in the isolated yields (70% and 57%) is likely a result of varying the scale of the reaction. When **1.94** is isolated in mg amounts, it often forms as oil which can be difficult to fully purify. With larger scale reactions, sufficient material is isolated to allow crystallization, which is more facile on a larger scale, so that the 57% isolated yield of **1.94** represents a robust practical measure of the success of the reaction.

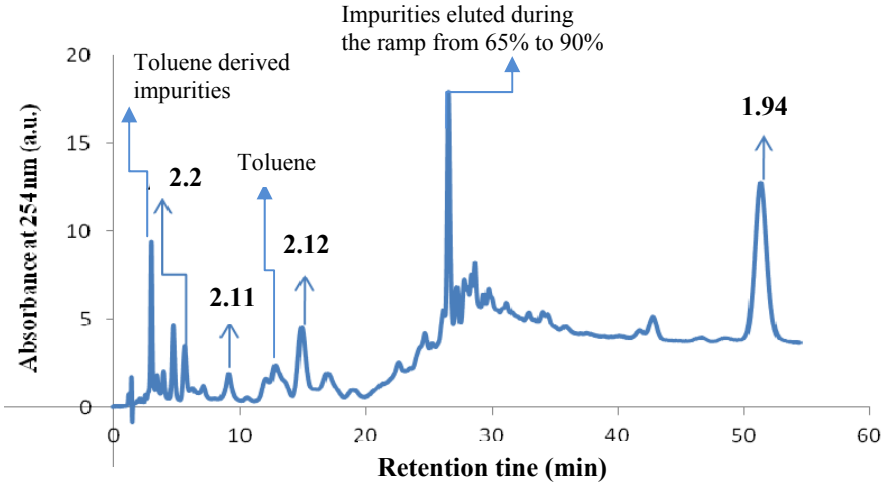
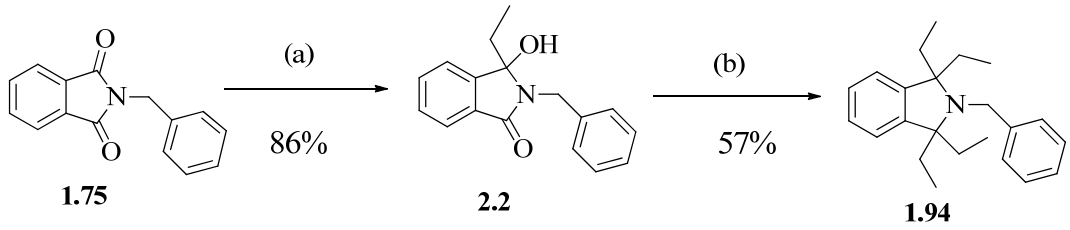


Figure 2.3 HPLC chromatogram of the reaction mixture of Grignard tetraethylation of **2.2** (Elution method: See Figure 2.2).

(**Elution method**: see Figure 2.2)

Toluene and toluene derived impurities were identified in the chromatograms (Figure 2.2 and 2.3) by evaporating some amount of toluene (same toluene used for the Grignard reactions) and analysing the remaining residue by HPLC under the same elution conditions stated above in Figure 2.3. Finally the overall yield of **1.94** was improved to 49% over two steps (Scheme 2.11) using this approach, which represents a higher overall yield compared to the single step synthesis by Caldararo *et al.*[110] (41%).



Overall yield of **1.94** over two steps = 49%

Scheme 2.11 Improved synthetic route and the overall yield of **1.94** over two steps.

Reagents and conditions: (a) EtI (2.5 equiv), Mg (2.5 equiv), Et_2O, Ar, rt, 1 h, **1.75** (1.0 equiv.), toluene, 86%; (b)) EtI (6.0 equiv), Mg (8.0 equiv), Et_2O, Ar, rt, then toluene reflux, 72 h with **2.2** (1.0 equiv), 57%.

Initially it was speculated that the significantly improved yield of tetraethyl adduct **1.94** obtained from ethylhydroxy amide **2.2** (Scheme 2.11) was probably due to the different work-up methodology followed to analyse the reaction mixture of the respective reaction. The standard Grignard tetraethylation of *N*-benzylphthalimide **1.75** was therefore driven under the experimental conditions similar to the Grignard ethylation of **2.2** (conditions of Entry 3, Table 2.6) using a similar work-up (involving aqueous NH_4Cl) to investigate the impact of the work-up methodology on the overall yield of tetraethyl adduct **1.94**. This reaction demonstrated a comparatively lower relative HPLC yield for **1.94** (Entry 1, Table 2.7) starting from **1.75**, indicating that the work-up may have a limited impact on the improved yield of **1.94** when the Grignard ethylation was undertaken on **2.2**.

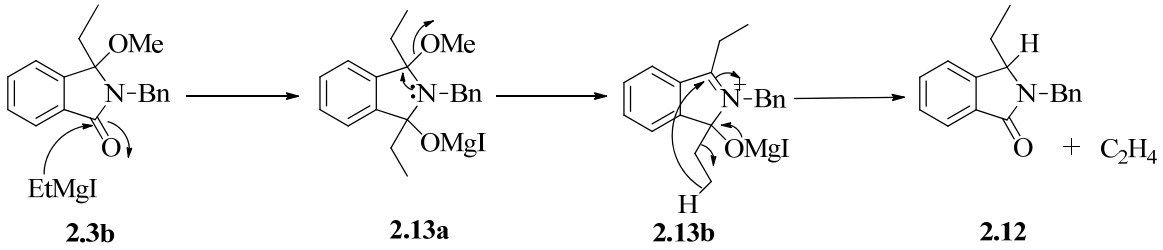
Table 2.7 HPLC product ratios obtained from the Grignard tetraethylation of **1.75**

Entry	Reaction temperature (°C)	Reaction time (hrs)	Equiv. EtI	Equiv. Mg	HPLC product ratio (%) **1.94**	**2.11**	**2.12**	**2.2**
1	110	72	6	8	24	22	1	53
2	110	96	6	8	35	16	3	46

2.3.4.3 Mechanistic investigations of the reactions to form **1.94**, **2.11**, **2.12** and **2.2**

The monoethyl amide **2.12** is unusual as it contains a H atom and so formally represents a reduction of the starting material. Two mechanisms were proposed for the formation of **2.12** and some experiments were carried out to elucidate the mechanisms involved.

Mechanism 1



Scheme 2.12 Proposed mechanism for the formation of **2.12**.

To test the proposed mechanism shown above (Scheme 2.12), 1.5 equivalents of EtMgI were reacted with **2.3b** at room temperature for 2 hours, and then the reaction mixture was analysed by TLC. Since the substrate remained in the reaction mixture after 2 hours, the temperature of the reaction mixture was increased to 40 °C (for 24 hours), 50 °C (10 hours) and 60 °C (10 hours); however, no new products were observed by TLC. The absence of any new components in the reaction mixture prompted us to reflux the mixture at 110 °C for 3 hours. However, the analysis of the reaction mixture by HPLC (Figure 2.4) again showed the presence of unreacted starting material (95%) even after heating at reflux for 3 hours.

By considering all these results, finally mechanism 1 (Scheme 2.12) was ruled out and a new mechanism was suggested for the formation of product **2.12** as shown in Scheme 2.13 below.

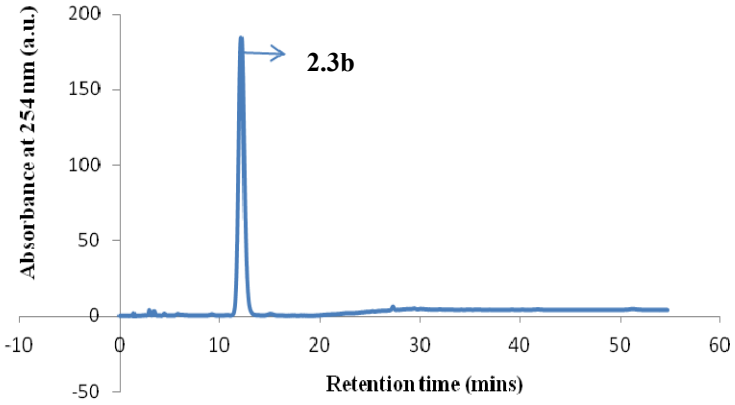
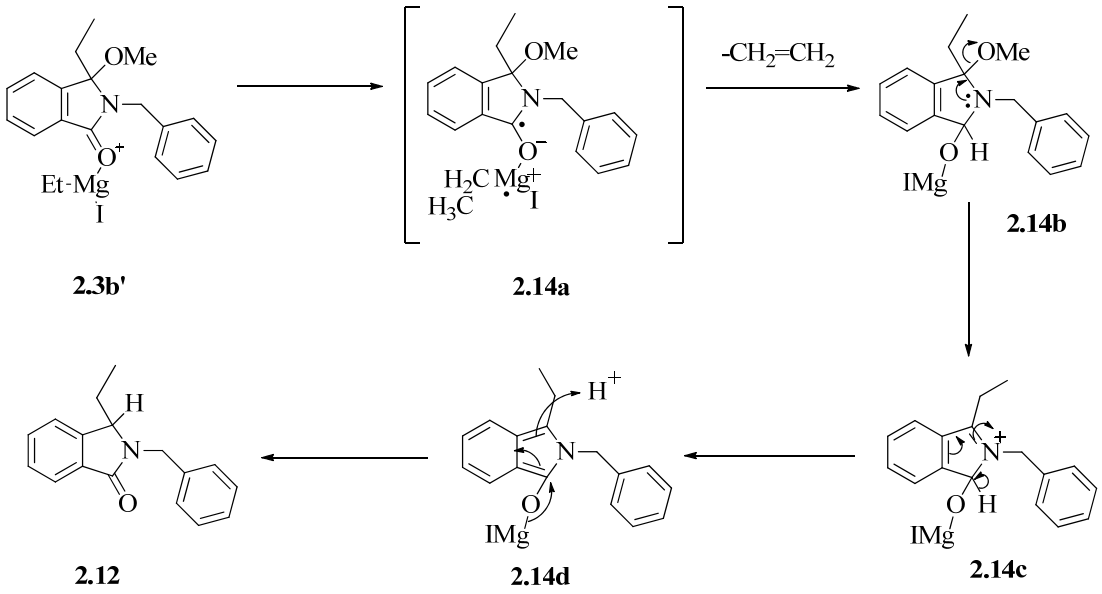


Figure 2.4 HPLC chromatogram of the reaction mixture of **2.3b** with 1.5 equivalents of EtMgI after stirring at several different reaction temperatures.

(**Elution method**: See Figure 2.2)

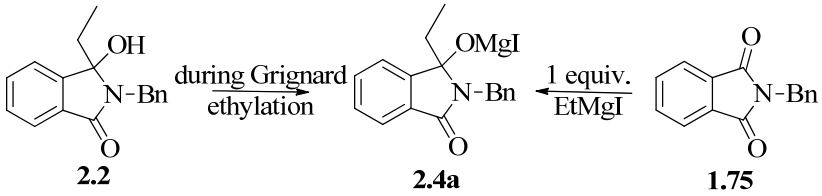
Mechanism 2



Scheme 2.13 Proposed mechanism for the formation of **2.12**.

The mechanism shown in Scheme 2.13 was proposed by inference from two mechanisms documented in the literature.[137,138] According to these established mechanisms, one electron is transferred to **2.3b'** from EtMgI by a single electron transfer mechanism to form the radical anion-radical cation pair **2.14a**. The rate of

this single electron transfer is dependent on the number of β-hydrogen atoms present in the alkyl group of the Grignard reagent.[138] This single electron transfer occurs much faster with ethyl Grignard reagents, since these contain three β-hydrogen atoms.[138] After forming radical cation-radical anion pair **2.14a**, β-hydrogen transfer may occur from the ethyl group of the Grignard reagent (by extrusion of one equivalent of ethene) to the carbonyl carbon of the molecule. This β-hydrogen atom transfer is dependent on the steric requirements of the alkyl group of the Grignard reagent.[138] Thus the ethyl group, which is less hindered than other alkyl Grignard reagents such as isobutyl, isopropyl, hexenyl, tertiary butyl *etc*., would give the reduction product **2.14b** at a much faster rate.[138] Finally, tautomerisation of **2.14d** would lead to the thermodynamically more stable amide **2.12**.[137]



2.2 **2.4a** **1.75**

Scheme 2.14 Formation of alkoxymagnesium salt **2.4a** starting from **1.75** and **2.2** during the Grignard ethylation.

According to Mechanism 2, formation of **2.12** could take place only with Grignard reagents having β-hydrogen atoms. Therefore **2.3b** was treated with 6 equivalents of MeMgI in refluxing toluene for 14 hours, and the mixture was analysed by HPLC (Figure 2.8). The chromatogram showed the formation of only trace amounts of **2.12** in the reaction mixture, which probably arises due to the presence of traces of iodoethane in commercially sourced iodomethane. This mechanism can also be used to explain the formation of **2.12** from both *N*-benzylphthalimide **1.75** and hydroxylamide **2.2** *via* alkoxymagnesium salt **2.4a** (Scheme 2.14). The structure of monoethyl amide **2.12** was supported by ^{1}H-NMR spectroscopy (Figure 2.5) due to

the appearance of two separated doublets for the benzylic methylene protons due to their relationship with the chiral centre at hydrogen/ethyl substituted ring position.

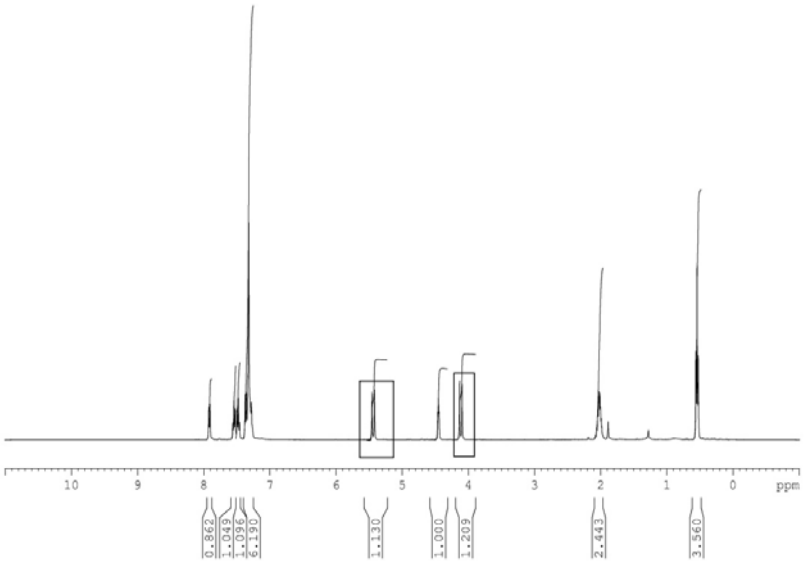
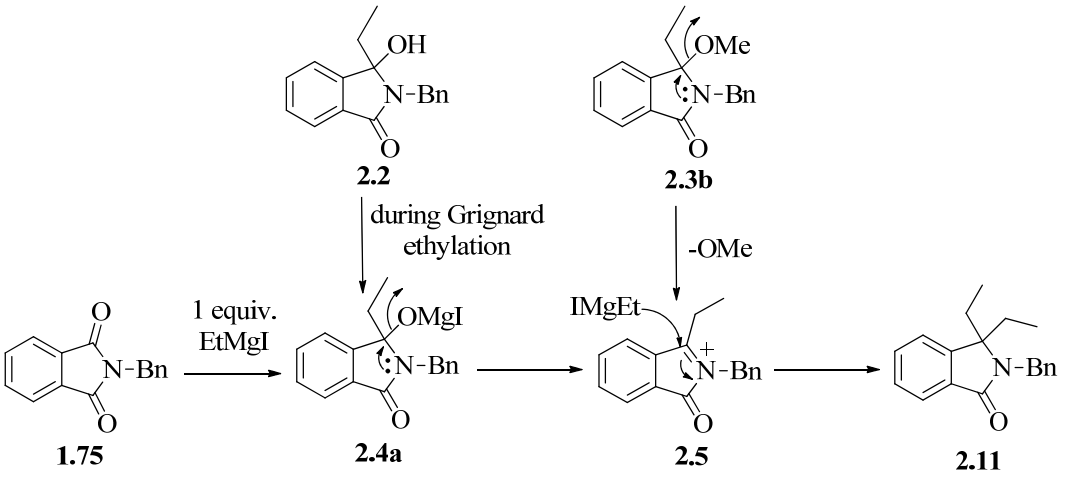


Figure 2.5 Appearance of benzylic methylene protons as two doublets in the ^{1}H-NMR spectrum of **2.12**.

The second product **2.11** formed in this reaction was also interesting, as it was hitherto unreported. This product was found to be generated commencing from three different substrates: methoxyamide **2.3b**, hydroxylamide **2.2** and *N*-benzylphthalimide **1.75**. The highest relative HPLC yield (29%) for diethyl amide **2.11** was obtained starting from methoxyamide **2.3b** (Entry 2, Table 2.5). This implies that the intermediate, which could lead to **2.11**, might have generated in a higher level when the reaction was started from **2.3b**. The intermediate which gives rise to **2.11** during the reaction could possibly be the iminium ion **2.5**, as such ions likely form faster due to the presence of a better leaving group (-OMe). When the ethylation was performed starting from the hydroxylamide **2.2**, the HPLC product ratio obtained indicated less **2.11** (9%, Entry 5, Table 2.6 *vs* 26%, Entry 7, Table 2.5) was formed compared to when the alkylation was undertaken using **2.3b**. The lower

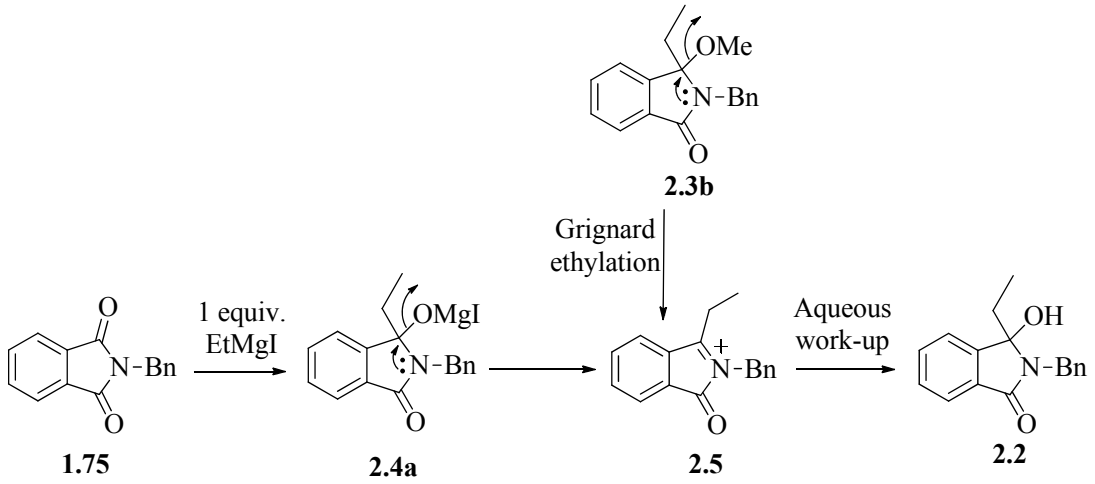
yield of **2.11** in this reaction may be due to the relatively poor leaving group (-OMgI) shown in structure **2.4a** compared to the better leaving group (-OMe) in **2.3b** (Scheme 2.15). This explanation however leads to a conclusion that **2.11** could possibly form from iminium ion **2.5** during the ethylation.



Scheme 2.15 Proposed mechanism of forming **2.11**, starting from **1.75**, **2.2** and **2.3b**.

Ethylhydroxyl amide **2.2** was another product observed in both the Grignard ethylation of methoxy amide **2.3b** and phthalimide **1.75**. This hydroxyamide **2.2** would be expected to arise from the initial precursor intermediate **2.5** or hydrolysis of the alkoxy magnesium salt **2.4a** during the aqueous work-up. This was concluded as there were no any hydroxyl ions or H^+ ions in the Grignard reaction mixture, either to attack iminium ion **2.5**, or to protonate the alkoxy salt **2.4a**, to give rise to hydroxyl amide **2.2**. On the other hand, when both **2.2** and phthalimide **1.75** were treated with excess EtMgI, about 80% relative HPLC yield (Entry 5, Table 2.6) and 24% relative HPLC yield (Entry 1, Table 2.7) were given for tetraethyl adduct **1.94** respectively. From this comparison, it was deduced that ethylhydroxy amide **2.2** is generated on work-up from iminium ion **2.5**. If it is generated in the form of **2.2**, we would have expected to see an improved yield for tetraethyl adduct **1.94** in Entry 1 (Table 2.7). Formation of a modarate amount of ethylhydroxy amide **2.2** and a higher

amount of diethylamide **2.11** (Table 2.5) during the ethylation of **2.3b** suggests the influence of an improved leaving group strategy.

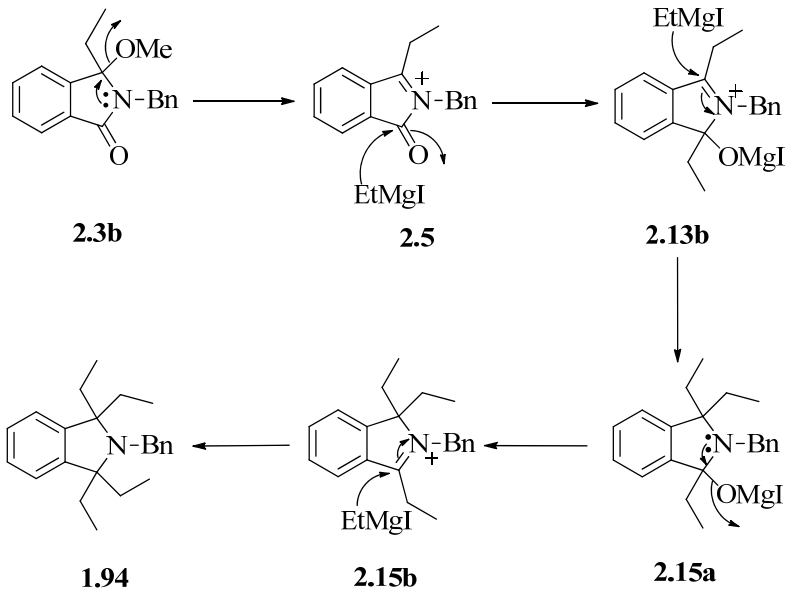


Scheme 2.16 Proposed mechanism for the formation of ethylhydroxy amide **2.2**, starting from **2.3b** and **1.75**.

The mechanism of the formation of 2-benzyl-1,1,3,3-tetraalkylisoindoline during the tetraalkylation of **1.75** was first reported by Braslau (Scheme 1.24, Chapter 1)[108] by synthesizing regiospecific *N*-benzyl-1,3-dimethyl-1,3-diphenylisoindoline **1.86b**. A similar mechanism can be used to explain the formation of **1.94** starting from **2.3b** as well. Due to the improved leaving group strategy, tetraethyl adduct **1.94** would be expected to be generated from **2.3b** in a higher yield compared to that from phthalimide **1.75**. The experimental observations were, however, contrary to this expectation, due to the formation of two dead-ends, diethyl amide **2.11** and monoethyl reduction product **2.12**.

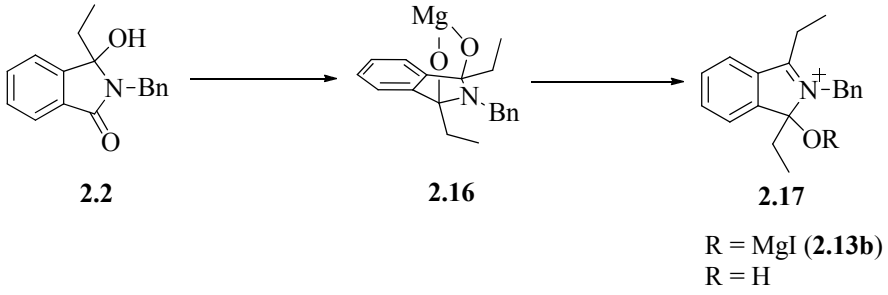
When tetraethyl adduct **1.94** was synthesized from ethylhydroxy amide **2.2**, the relative HPLC yield of **1.94** (80%) was significantly improved, and the yield of side-product **2.11** was lower (9%, Entry 5, Table 2.6) compared to when the ethylation was undertaken using **2.3b**. This implies that starting the reaction by adding the ethyl Grignard reagent to the ethylhydroxy amide **2.2** may enable an additional pathway to

that which occurs when starting from either *N*-benzylphthalimide **1.75** or methoxy amide **2.3b**.



Scheme 2.17 Accepted mechanism for the formation of **1.94**, starting from **2.3b**.

It was proposed that formation of **1.94** starting from **2.2** might take place through a different intermediate such as **2.16** (Scheme 2.18), in which iminium ion **2.5** is unlikely to be involved. Established literature[136] supports that bridging complexes such as **2.16** have been suggested to play important roles in some Grignard reactions.



Scheme 2.18 Proposed mechanism of formation of **1.94** via O-Mg-O bridging complex (**2.16**) commencing from ethylhydroxy amide **2.2**.

Formation of such a brdging complex (**2.16**) may efficiently produce an intermediate like **2.17** (Scheme 2.18) which is believed to appear further along in the reaction pathway of tetraethyl adduct **1.94**. Intermediate **2.17** could eventually convert to **1.94**

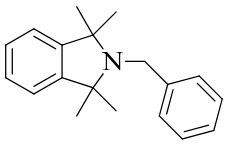
in a significantly higher yield. This proposed mechanism for the formation of **1.94** starting from **2.2** explains why the lower levels of **2.11** were detected in the reaction mixture.

2.4 CONCLUSIONS

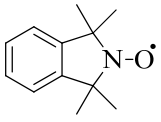
The exhaustive Grignard ethylation of phthalimide **1.75** gives 1,1,3,3-tetraethylisoindoline **1.94** in an atom-efficient, single reaction. This is nonetheless a low yielding process (28-40%). Identification and subsequent investigation of various side-products generated in the reaction (**2.11**, **2.12** and **2.2)** indicated that there are a number of species generated that do not react further to give rise to the final target product. The aim of this study was to investigate the influence of improved leaving groups on the product yield. The introduction of better leaving groups favoured a new reaction process to give exocyclic amide **2.6**. The use of a methoxy ether **2.3b** as the leaving group decreased elimination, but favoured the formation of increased amounts of diethyl amide **2.11**, which does not undergo further ethylation. Using the methoxy ether **2.3b** as the starting material gave rise to significant levels of the hydroxyl amide **2.2**, presumably formed from the iminium ion **2.5** on aqueous work-up. Surprisingly, high levels of iminium ion do not promote the formation of tetraethyl target (**1.94**), indicating greater complexity in the reaction mechanism than has been previously recognised. When the overall yield of **1.94** was calculated over three steps (Scheme 2.10) *via* methoxy amide **2.3b**, it was 41%, which is similar to the current isolated yield of **1.94** over one step.

Attempts to further alkylate diethyl amide **2.11** to generate tetraethyl adduct **1.94** were not successful, presumably because steric hindrance limits further ethylation upon the ring. Treatment of monoethyl reduction product **2.12** with excess EtMgI does not lead to **1.94**, thus both **2.11** and **2.12** were recognized as “dead-end” side

products of the reaction. However, reacting hydroxyl amide **2.2** with 6 equivalents of EtMgI surprisingly gave improved yields for **1.94** on both small scale (70% isolated yield) and large scale (57% isolated yield). When the overall yield of **1.94** was calculated (Scheme 2.11), it was improved compared to the previous one-pot procedure. This new approach provides a practical preparative scale improvement over the existing synthesis. Starting from hydroxyl amide **2.2** may allow the formation of Mg salt complexes such as the bridging complex **2.16**, which may follow to Mg salt species such as **2.17** which are necessary for the formation of tetraethyl adduct **1.94**. In the mechanistic investigations of the reactions that generate the side-products, a product (**2.12**) was identified which might be generated by the reduction of amide carbonyl of the starting material used (Scheme 2.14).[138] The formation of diethyl amide **2.11** presumably occurs via attack of EtMgI on the iminium ion **2.5** (Scheme 2.15 and 2.16), and formation of the hydroxyl amide **2.2** most likely occurs via the hydrolysis of either the iminium ion **2.5** or the corresponding magnesium alkoxide **2.4a** during the aqueous work-up (Scheme 2.17 and 2.18).



1.76



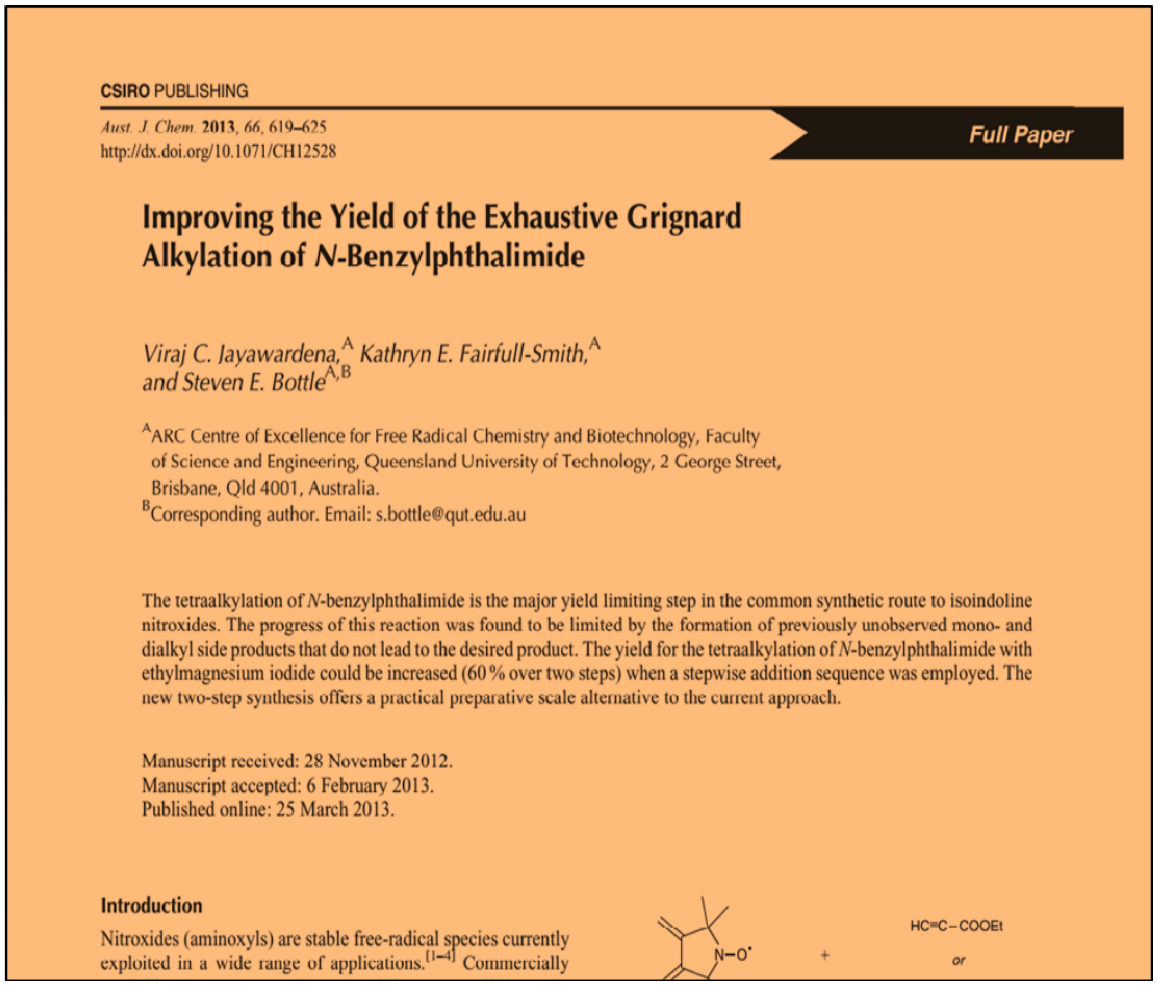
1.15

Figure 2.6 Structures of 1,1,3,3-tetramethyl-2-benzylisoindoline **1.76** and its nitroxide, TMIO (**1.15**)

This project resulted in an improvement in the yield of the isoindoline nitroxide TEIO (**1.13**) up to 40% (from 33%) from phthalimide **1.75**.[88] Since improving the yield of tetraethyl adduct **1.94** succeeded with the stepwise addition method, improving the yield of tetramethylisoindoline analogue **1.76** will be focussed upon

next. However, it has long been known that the methylation of phthalimide **1.75** is problematic and low-yielding compared to tetraethylation due to the formation of large amounts of an unknown purple-coloured oligomeric material. Therefore, the following chapter will deal with improving the yield of **1.76** by further investigation of the exhaustive Grignard methylation reaction.

2.5 PUBLICATION DERIVED FROM THE WORK DESCRIBED IN CHAPTER 2



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Full Paper

Improving the Yield of the Exhaustive Grignard Alkylation of *N*-Benzylphthalimide

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The tetraalkylation of *N*-benzylphthalimide is the major yield limiting step in the common synthetic route to isoindoline nitroxides. The progress of this reaction was found to be limited by the formation of previously unobserved mono- and dialkyl side products that do not lead to the desired product. The yield for the tetraalkylation of *N*-benzylphthalimide with ethylmagnesium iodide could be increased (60% over two steps) when a stepwise addition sequence was employed. The new two-step synthesis offers a practical preparative scale alternative to the current approach.

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Introduction

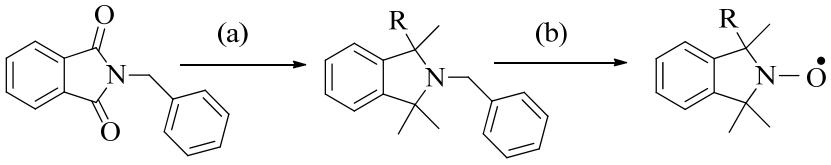
Nitroxides (aminoxyls) are stable free-radical species currently exploited in a wide range of applications.[1–4] Commercially

(Full paper is attached in the Appendices)

Chapter 3: Further Insights into the Exhaustive Grignard Tetramethylation of *N*-Benzylphthalimide

3.1 BACKGROUND

1,1,3,3-Tetramethylisoindolin-2-yloxyl (TMIO, **1.15)** is a versatile radical scavenger owing to useful features, such as: Thermal stability, non-reactivity towards olefins,[87] symmetrical nature,[35] UV detectability,[62] and relative inertness to free radical attack by oxygen radicals.[35] The most common and effective pathway for synthesizing TMIO remains a Grignard-based approach in which *N*-benzylphthalimide **1.75** is treated with methylmagnesium halide in refluxing toluene, followed by subsequent debenzylation and oxidation of the resulting secondary amine (Scheme 3.1).[35] The overall yield of TMIO (**1.15**) from this approach, however, is modest (~30%), due to the crucial Grignard reaction being low yielding.



1.75 **(1.82) R = Et** **(1.76) R = Me** **(1.81) R = Et** **(1.15) R = Me**

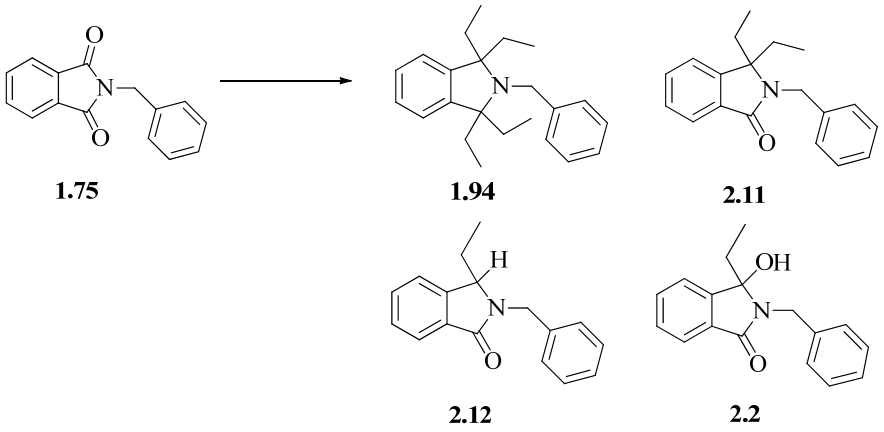
Scheme 3.1 Generation of TMIO via the addition of MeMgI to *N*-benzylphthalimide.

Reagents and conditions: (a) MeI (6 equiv), Mg (6 equiv), Et_2O, N_2, rt, Toluene reflux, 4 h, **1.75** (1.0 equiv), 37%; (b) (i) **1.76** in AcOH, H_2/Pd/C, rt, 3 h, 60 lb/in^2, then NaOH (10%), Et_2O, 96%; (ii) Secondary amine in MeOH/CH_3CN, then $NaHCO_3$ (1.0 equiv), $(NH_4)_6Mo_7O_{24}.4H_2O$ (0.009 equiv), then H_2O_2 (30%, 4.0 equiv), rt, 32 h, 92%.[35]

The methylation of *N*-benzylphthalimide typically proceeds in yields of 28-37%. Various attempts have been made to improve the yield of this reaction, such as

Scammells' method (45% yield)[111] and Caldararo's method (28% yield).[139] Although the first method improved the yield of tetramethyl adduct **1.76** (to 45%), it is not practical for large-scale reactions.[111] Performing the methylation reaction on a modified substrate (*N*-methylphthalimide **1.78**) was not effective, due to the unavoidable formation of 2-methyl-1-ethyl-1,3,3-trimethylisoindoline **1.80** (Figure 1.10) which could not be totally eliminated by standard purification procedures, nor avoided by varying the reaction conditions;[35] another problem was the unexpected ring opening of **1.78** under microwave conditions.[111]

Notably, in the organic synthesis reported by Griffiths and co-workers in 1983, an unwanted side product, 2-benzyl-1-ethyl-1,3,3-trimethylisoindoline (**1.82**) was detected, along with the main tetramethyl adduct **1.76**.[35] Although it was not possible to eliminate the formation of this side product by chromatography, it could be separated from the major product by recrystallization. Furthermore, a petroleum ether-insoluble, purple-coloured intractable material was also generated during the reaction.[35] The formation of these unwanted products may be a reason for the low yield of the target, 2-benzyl-1,1,3,3-tetramethylisoindoline **1.76**. No well-defined products apart from **1.82** and **1.76** could be isolated from the reaction mixture. However, the yield of **1.76** indicates that other side products must be formed in the reaction. As discussed in Chapter 2, Grignard ethylation of *N*-benzylphthalimide **1.75** potentially gives rise to alkylated products: diethylamide **2.11** and monoethyl reduction product **2.12**.[140] Mechanistic experiments demonstrated that both **2.11** and **2.12** were not intermediates in the pathway to the tetraethyl adduct **1.94**.



Scheme 3.2 Products generated by tetraethylation of phthalimide **1.75**[140]

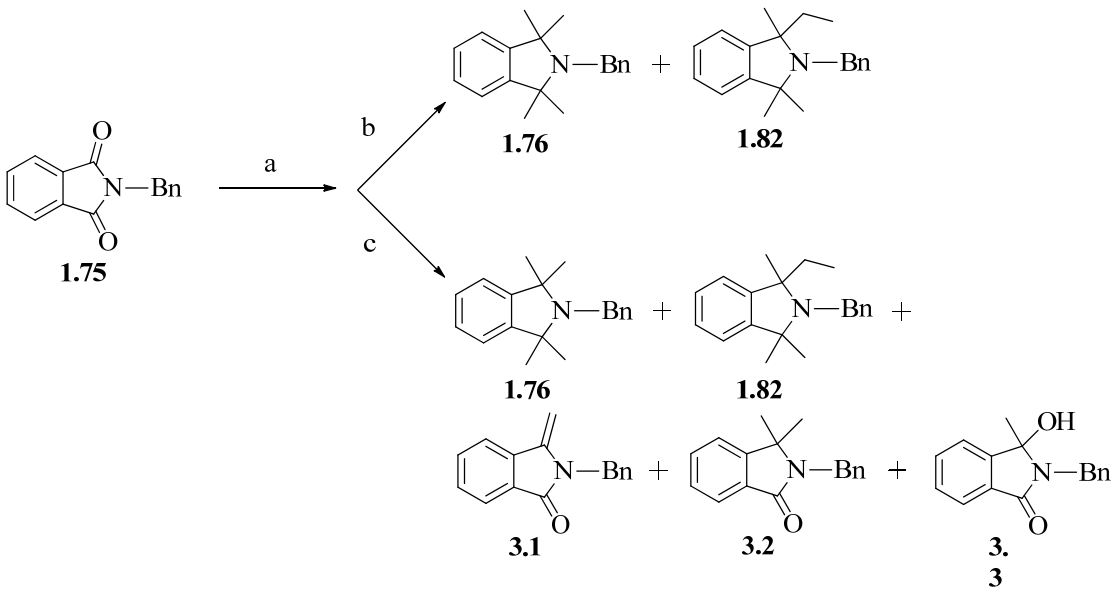
Reagents and conditions: (a) **1.75** (1.0 equiv.), EtMgI (6.0 equiv.), toluene, 110 °C, 72 h; **1.94** (24%), **2.11** (22%), **2.12** (1%), **2.2** (53%).[140]

Based on these findings, we decided to re-investigate the methylation of *N*-benzylphthalimide in order to determine if similar and hitherto unreported side products are formed. An improved mechanistic understanding of this reaction could provide a rational basis for improving the yield by modifying the conditions.

3.2 RESULTS AND DISCUSSION

According to the original Griffiths procedure, refluxing **1.75** with excess MeMgI (MeMgI was used for the tetramethylation of **1.75** instead of MeMgBr, due to its higher reactivity) in toluene gave only two products: tetramethyl adduct **1.76** (37%) and ethyltrimethyl adduct **1.82** (0.03%), after extracting the Grignard reaction mixture with hexane followed by a celite filtration and basic alumina column chromatography.[35] No other well defined products other than **1.76** and **1.82** were observed. In our hands, performing a Grignard methylation on phthalimide **1.75** gave 27% of **1.76** and 3% of **1.82** without any other notable products in the organic layer. Notably this reflects a significant loss of starting material (about 70%) that is not converted to the adducts **1.76** and **1.82**. Consequently a different work-up for the reaction was employed.

After heating phthalimide **1.75** with MeMgI at reflux for 4 hours, the reaction mixture was quenched by NH_4Cl and the toluene layer was separated. The aqueous NH_4Cl layer was extracted with chloroform and the combined chloroform and toluene fractions were evaporated. The products obtained from the organic layers were analysed by HPLC. This revealed the presence of five different products. Isolation of the products and analysis by NMR spectroscopy indicated that these compounds were enamide **3.1**, dimethyl amide **3.2**, and methyl-hydroxy amide **3.3**, in addition to the expected adducts **1.76** and **1.82** (Scheme 3.4, path c). Yields of these products are given in Table 3.1 below.



Scheme 3.4 Reaction products isolated from the methyl Grignard reaction of *N*-benzylphthalimide **1.75** (dependant on work-up).

Reagents and conditions: (a) MeMgI (6.0 equiv.), Toluene, 110 °C, 4 h ; (b) Reaction work-up by extraction of the reaction mixture into hexane, filter through celite, aerial oxidation of the filtrate, and eluting through basic alumina; (c) Reaction work-up by quenching the reaction mixture with NH_4Cl, separation of the toluene layer, extraction of the aqueous NH_4Cl layer to $CHCl_3$, combination of organic layers.

Presumably these three new previously unreported products (**3.1**, **3.2** and **3.3**) were lost during the neutral alumina work-up used in the Griffiths' procedure. Further

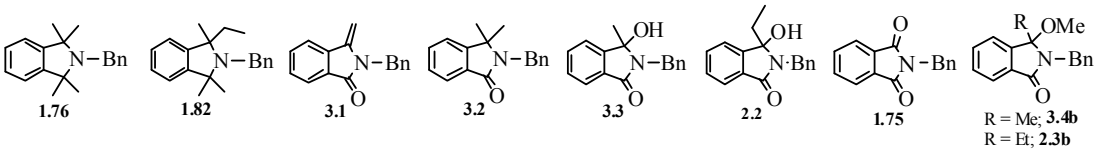
investigations were carried out to explore the potential pathways leading to these side-products to determine if they could be influencing the overall yield of tetramethyl adduct **1.76** formed in the reaction.

When the isolated % yields of the five products obtained from NH_4Cl work-up (Table 3.1, Entry 1) were analysed, the product formed in the highest yield was the hydroxymethyl adduct **3.3**. Previously, we speculated that an ethylhydroxy amide product such as **2.2** could be generated from an iminium ion during the aqueous work-up.[140] Presumably, methylhydroxy amide **3.3** is generated on aqueous work-up from the iminium ion **3.5**, or from the magnesium salt intermediate **3.4a**, each of which would arise from the single alkylation of phthalimide starting material **1.75**. This intermediate (**3.4a**) represents the key initial step on the synthetic pathway to the desired tetramethyl adduct **1.76** (Scheme 1.24). Therefore, it would be expected that increasing the reaction time is necessary to drive the reaction beyond a single alkylation. Extending the reflux time of tetramethylation of phthalimide **1.75** from 4 hours to 72 hours decreased the level of methylhydroxyamide **3.3**, and increased the isolated amounts of ethyltrimethyl adduct **1.82**, alkene **3.1** and diethylamide **3.2**. However, there was only a minor increase in the yield of tetramethyl adduct **1.76** (Table 3.1, entry 2). Raising the reaction temperature from 110 °C to 140 °C by replacing toluene with xylene similarly decreased the amount of methylhydroxy amide **3.3** detected, but did also not improve the yield of tetramethyl adduct **1.76** (Table 3.1, entry 3). Therefore, it was decided to explore the roles of **3.3** and the other products generated during the synthesis of tetramethyl adduct **1.76**. In this regard, methylhydroxy amide **3.3** was synthesized in order to use this species as a starting material in the methyl Grignard reaction.

Table 3.1 Isolated % yields of products obtained during tetramethylation of different substrates in refluxing toluene.

Entry	Reaction temp[°C]	Reaction time [h]	Equiv. Mg	Equiv. MeI	Isolated % yields **1.76**	**1.82**	**3.1**	**3.2**	**3.3**	**2.2**	**1.75**
1[A]	110	4	8.0	6.0	27	3	4	7	33	-	-
2[A]	110	72	8.0	6.0	31	5	7	16	4	-	4
3[A]	140	4	8.0	6.0	25	1	2	10	4	-	2
4[B]	110	72	8.0	6.0	20	5	11	6	3	2	3
5[C]	110	72	8.0	6.0	-	5	-	3	6	1	8
6[D]	110	72	8.0	6.0	36	1.5	1	13	6	1	2
7[E]	110	72	8.0	6.0	-	30	-	4	3	10	3
8[F]	110	72	8.0	6.0	-	29	3	4	6	11	8

[A] Using phthalimide **1.75** as starting material/ [B] Using methylhydroxy amide **3.3** as starting material/ [C] Using exocyclic alkene **3.1** as starting material/ [D] Using methylmethoxy amide **3.4b** as starting material/ [E] Using ethylhydroxy amide **2.2** as starting material/ [F] Using ethylmethoxy amide **2.3b** as starting material.



1.76 **1.82** **3.1** **3.2** **3.3** **2.2** **1.75**
R = Me; **3.4b**
R = Et; **2.3b**

3.2.1 Synthesis of 2-benzyl-3-hydroxy-3-ethylisoindolin-1-one **3.3**

3.2.1.1 Background

Compound **3.3** was synthesized by Igawa and co-workers in 2006, with a yield of 92% by reacting *N*-benzylphthalimide **1.75** with 1.0 equivalent of MeLi in THF at -78 °C.[141] However, the full experimental details of the synthetic procedure or the characterization of the compound **3.3** were not reported.

3.2.1.2 Synthesis and optimization of **3.3**

Following the method developed for the ethyl analogue (**2.2**, Scheme 3.2) of **3.3** described in Chapter 2, the synthesis of **3.3** was achieved by reacting *N*-benzylphthalimide **1.75** with 2.5 equivalents of MeMgI at room temperature for 2 hours. Followed by an aqueous NH_4Cl work-up, extraction of the aqueous layer with chloroform and combination with the original toluene layer gave off-white coloured crude on evoporation. Recrystallization of this crude material from toluene gave exocyclic alkene **3.1** (85%) as the major product, while the mother liquor contained a small amount alcohol of **3.3** (10%) as the minor product (Table 3.2, Entry 1). Three reasons were assumed for the conversion of **3.3** to **3.1**:

1. The three hour duration of the reaction at room temperature may be too long for a single methylation;
2. The quenching of the reaction mixture with slightly acidic NH_4Cl may lead to elimination;
3. Dehydration of methylhydroxy amide **3.3** during recrystallisation.

When the reaction mixture was stirred for only one hour at room temperature (Table 3.2, entry 2) followed by an aqueous NH_4Cl work-up, the NMR spectrum of the crude (before recrystallization) showed a clean spectrum of **3.3** in 90% yield with 98% purity by HPLC. However, recrystallization of this crude from toluene again gave alkene **3.1** as the major product. Recrystallization from hexane and ethyl acetate allowed the isolation of **3.3** in 63%; however, the mother liquor contained exocyclic amide **3.1** as a by-product (30%). As the crude reaction product was 98% pure by HPLC and the isolated yield of methylhydroxy amide **3.3** was above 90%, further experiments were pursued with crude **3.3**, unless stated otherwise.

Table 3.2 Isolated % yield of methylhydroxy amide **3.3** obtained during optimising reaction time and recrystallisation solvent.

Entry	Reaction Temp.(◦C)	Reaction time (h)	Equiv. Mg	Equiv. MeI	Isolated % yields			
					Before recrystallization		After recrystallization	
					3.3	**3.1**	**3.3**	**3.1**
1[A]	25	2	2.5	2.5	94	-	10	85
2[A]	25	1	2.5	2.5	93	-	11	83
3[B]	25	1	2.5	2.5	95	-	63	30

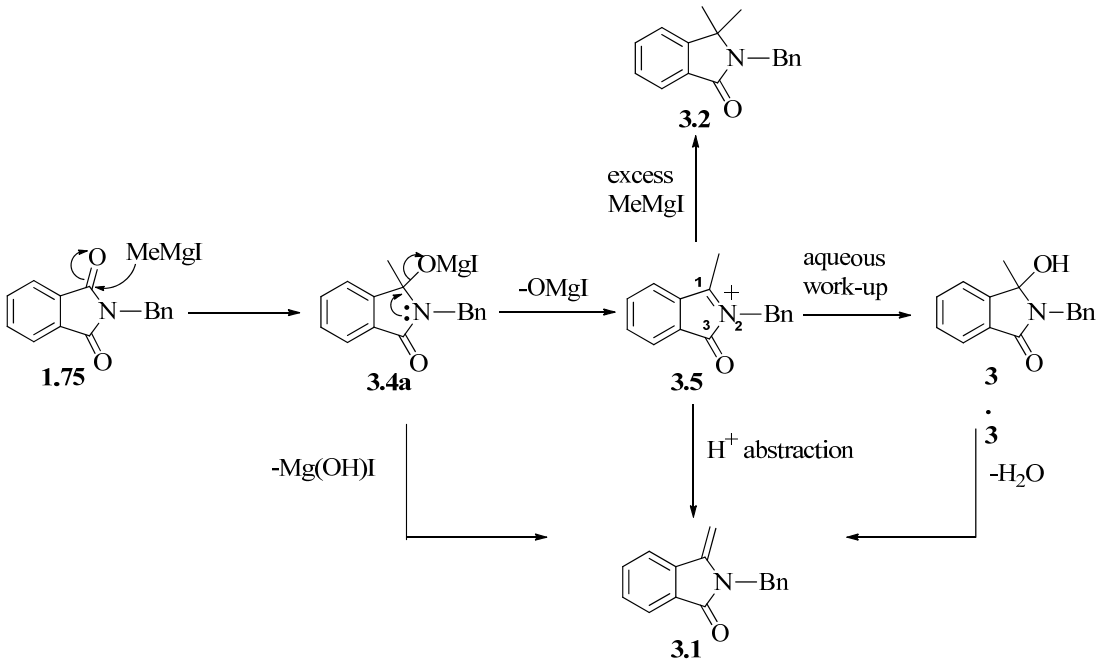
[A] Toluene for recrystallization/ [B] Hexane and ethylacetate for recrystallization

The observed differences in the recrystallised yields of **3.3** and **3.1** (Entry 2 & 3, Table 3.2) were mainly due to the solubility factor. Toluene was effective as a solvent to dissolve crude **3.3** only after heating toluene to 70-80 °C, where dehydration of crude **3.3** could be enhanced. In ethyl acetate, crude **3.3** was dissolved completely at comparatively lower temperatures (35-40 °C).

3.2.1.3 Exploring the Grignard methylation using methylhydroxy amide **3.3** as the starting material

Reacting product **3.3** with excess MeMgI in refluxing toluene for 72 hours gave six products, including ethyl hydroxy amide **2.2** and the reformation of *N*-benzylphthalimide **1.75** (Table 3.1, Entry 4). This reaction generated the desired tetramethyl adduct **1.76** as the main product in 20% isolated yield (Table 3.1, Entry 4). Comparison of the moderate yield of **1.76** (20%) generated using **3.3** as the starting material (Table 3.1, Entry 4) with the yield of **1.76** under the previously stated reaction conditions (Table 3.1, Entry 1 and 2) suggests **3.3** can be converted to the iminium ion **3.5** efficiently (*via* **3.4a)** during the Grignard reaction. (When the Grignard ethylation was performed on ethylhydroxy amide **2.2** in the previous chapter, it was hypothesized that the reaction is unlikely to go through the ethyl

iminium ion **2.5** due to the absence and lower levels of side-products which derived from iminium ion **2.5**).



Scheme 3.5 Formation of **3.1**, **3.2** and **3.3** during the tetramethylation of **1.75**.

Formation of the alkene **3.1** may occur through two possible pathways; one pathway may involve the spontaneous elimination of Mg(OH)I from alkoxide **3.4a**.[142] Alternatively, loss of a proton from the iminium ion **3.5** driven by the strongly basic Grignard conditions could also lead to this unwanted side-reaction. Gem-dimethyl amide **3.2** may arise due to the attack of MeMgI on **3.5** (addition at C_1) rather than the competing reaction of the addition to the other amide carbonyl group of **3.5** (addition at C_1 & C_3 alternatively). Due to the production of notable amounts of **3.1** and **3.2** starting from **3.3** (Entry 4, Table 3.1), it was concluded that **3.3** may largely go through intermediate **3.5,** rather than bridging complexes such as **2.16**. The precursor for the formation of all these side products, iminium ion **3.5**, has been proposed as the key intermediate in the synthetic pathway to give **1.76**.[16] However,

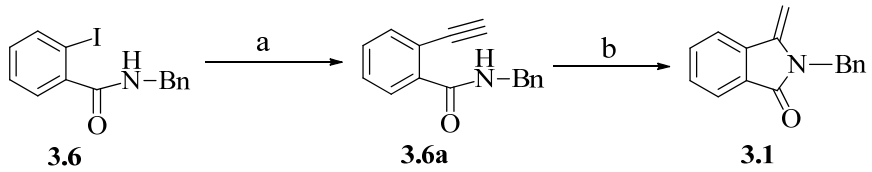
the side reactions of key intermediate **3.5 (**to give **3.1**, **3.2** and **3.3**) may represent a major reason for the poor yield of tetramethyl adduct **1.76**.

Therefore, it was decided to investigate the fates of the side products under these reaction conditions to determine which of them have an impact on the yield of tetramethyl adduct **1.76**. In this regard, it was of interest to know the fate of the exocyclic alkene **3.1** under the Grignard reaction conditions. Thus, an independant synthesis of alkene **3.1** was developed starting from methylhydroxy amide **3.3**.

3.2.2 Independant synthesis of 2-Benzyl-3-methyleneisoindolin-1-one **3.1**

3.2.2.1 Background

Compound **3.1** has been synthesized previously through palladium-catalysed Sonogashira coupling using *o*-iodobenzamides as the starting material.[143]



3.6 **3.6a** **3.1**

Scheme 3.6 Synthesis of **3.1** through a palladium-catalysed heteroannulation reaction.[143]

Reagents and conditions: (a)$HC\equiv CH$, $(PPh_3)_2PdCl_2$, CuI, Et_3N, DMF; (b) NaOEt/ EtOH

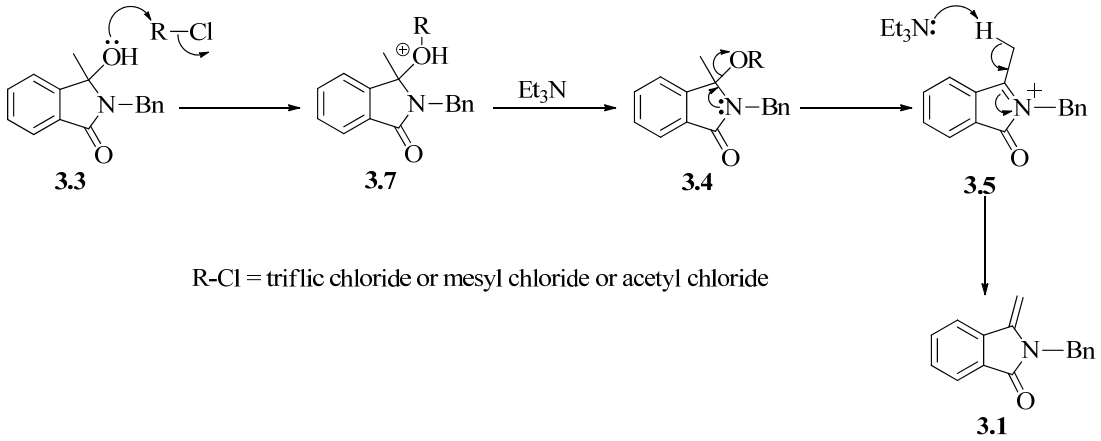
3.2.2.2 Synthesis and optimization of **3.1**

Synthesis of the alkene **3.1** was also achieved by the reaction of the hydroxyl amide **3.3** with three different acid chlorides. This was undertaken in an attempt to generate cyclic amides with good leaving groups, as discussed in Chapter 2. However, just as with the ethyl analogue, the methyl system exclusively gave the exocyclic alkene **3.1**.

Table 3.3 Isolated yields of alkene **3.1** obtained from **3.3** as the starting material.

Entry	Reaction temp. (°C)	Equiv. Acetyl chloride	Equiv. Et_3N	Reaction time (min.)	**3.1** yield (%)
1	0 - rt	1.2	1.2	30	83

As shown in Table 3.3, this proved to be a reasonably efficient means of generating **3.1**. The best isolated yield (83%) for **3.1** using this approach was obtained with acetyl chloride, which required a reaction time of 30 minutes.

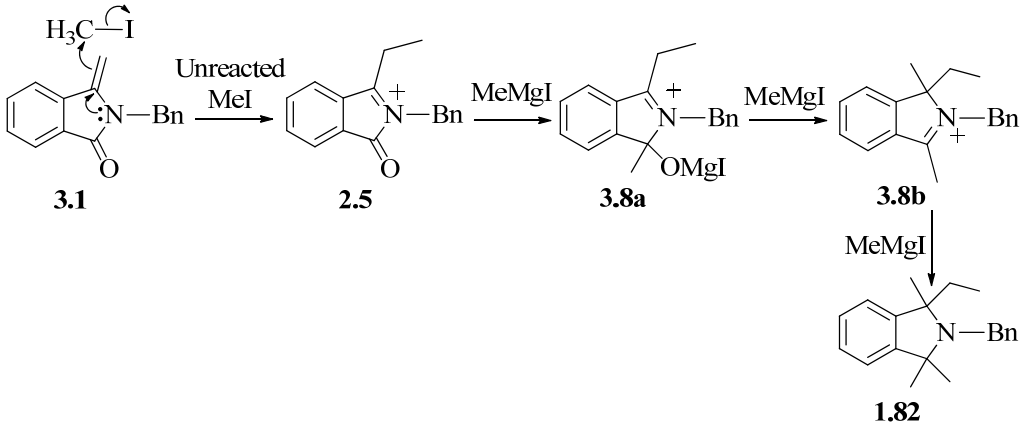


Scheme 3.7 Mechanism of formation of **3.1** through elimination reactions.

3.2.2.3 The further reaction of the alkene side-product **3.1** under Grignard conditions.

Treatment of **3.1** with excess MeMgI in refluxing toluene gave 5% of ethyl-trimethyl adduct **1.82** (Table 3.1, entry 5) along with dimethyl amide **3.2**, methylhydroxy amide **3.3**, ethylhydroxy amide **2.2** and phthalimide **1.75**. Notably, no desired tetramethyl adduct **1.76** was observed in the reaction mixture. As expected, alkene **3.1** does not appear to be an intermediate in the pathway to the desired tetramethyl adduct **1.76**, but it appears to be a key precursor for the formation of ethyl containing product **1.82**. It was speculated that product **1.82** might be formed by reacting **3.1**

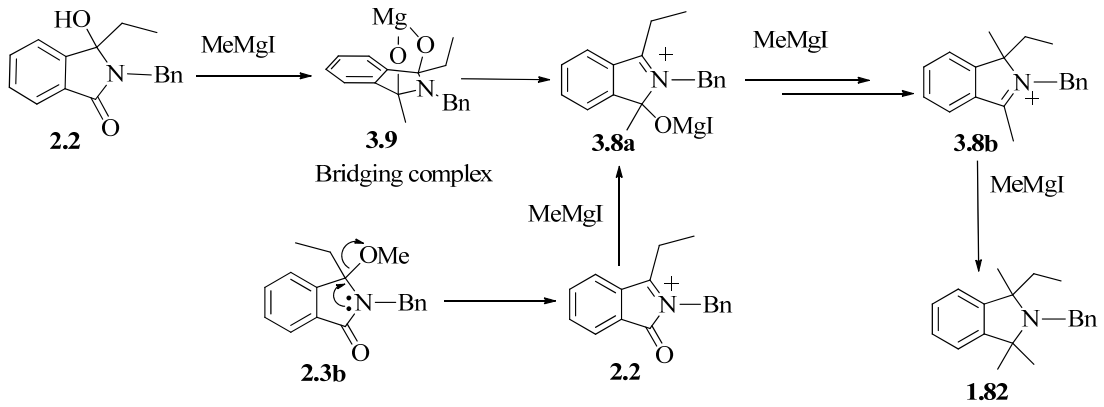
with unreacted MeI in the Grignard reagent to form iminium ion **2.5**, which may subsequently undergo methylation. In order to examine this, product **3.1** was subjected to two separate Grignard reactions, one with six equivalents of MeMgI containing excess Mg (8 equivalents) and the other with same equivalents of MeMgI containing excess MeI (8 equivalents). Both reactions were kept at 40 °C for 15 hours and then refluxed for further 72 hours. These two reactions were initially maintained at a low temperature (40 °C) to avoid the evaporation of excess MeI in the Grignard reagent. The mixture was then further refluxed at 110 °C to drive further methylation upon the assumption that iminium ion **2.5** has been formed in the reaction mixture. Analysis of these two reaction mixtures by HPLC indicated that the presence of excess MeI gave a significantly higher amount (35% yield) of ethyltrimethyl adduct **1.82** compared to the reaction with excess Mg, which would limit the amount of free MeI (20% yield).



Scheme 3.8 Proposed mechanism for the formation of **1.82** starting from **3.1**

According to the proposed mechanism for the formation of **1.82** shown in Scheme 3.8, product **3.1** could form an iminium ion **2.5** by nucleophilic attack on excess MeI in the reaction. This result is supported by the finding that in the original reported reaction conditions described by Griffiths *et al.*,[35] care must be taken to drive off ether (and any unreacted MeI) and raise the reaction temperature above 60 °C before

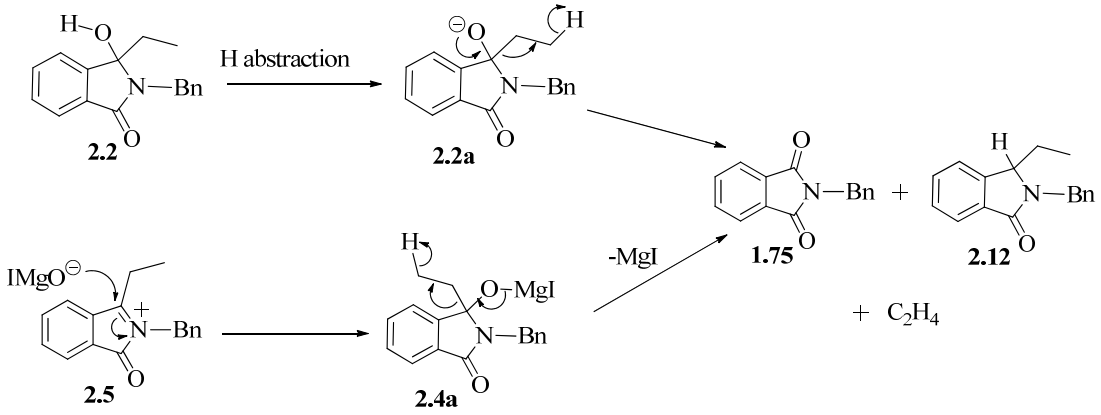
addition of the phthalimide, in order to minimise the formation of the mixed alkylation product **1.82**. However, it was predicted earlier that such iminium ions (**2.5**) may form the corresponding ethylhydroxide amide **2.2** during aqueous work-up.[140] This implies that treatment of ethylhydroxy amide **2.2**, or any other molecule which could produce iminium ion **2.5** during the Grignard reaction, would give rise to the increased amounts of **1.82** with excess MeMgI. As predicted, treatment of **2.2** with excess MeMgI gives increased yields of ethyltrimethyl adduct **1.82** (30%, Table 3.1, Entry 7) as the only tetraalkyl product, along with a small amount of reformed phthalimide **1.75** and some methyl substituted products: dimethyl amide **3.2** and methylhydroxy amide **3.3**. In addition, reacting ethyl methoxy amide **2.3b**, which should lead to higher levels of **2.5** due to the better leaving group,[140] with excess MeMgI also gave a considerably higher yield for **1.82** (29%, Table 3.1, entry 8). All these experimental results showed that product **1.82** could be formed from the further methylation of **2.5**, and that **2.5** could be formed by a nucleophilic addition of **3.1** with excess MeI as shown in Scheme 3.8. Undertaking the Grignard reaction on substrate that already possessed an ethyl group (**2.2** and **2.3b**) gave good yields of the ethyltrimethyl adduct **1.82**. These yields are higher than when the exocyclic alkene **3.1** is used as the starting material. This may be due to the ease by which each species can lead to an iminium ion such as **2.5**. However, the exocyclic alkene may be further reacting with the Grignard reagent to give an oligomeric/polymeric adduct as a competitive side reaction (See later).



Scheme 3.9 One proposed mechanism for the formation of **1.82** from **2.2** and **2.3b**.

The generation of *N*-benzylphthalimide **1.75** during the Grignard methylation reaction (Table 3.1, Entry 4-8) is both interesting and puzzling. Initially we speculated that **1.75** may be present in the reaction mixture as an unreacted substrate, but careful analysis of the starting material (by HPLC, ^{1}H-NMR spectroscopy and elemental analysis) showed that they did not contain any *N*-benzylphthalimide **1.75**. Notably, the highest yield for the regeneration of phthalimide **1.75** (8%) occurs with the methylation of **3.1** (Table 1, Entry 5) and of the ethyl methoxy amide **2.3b** (Table 1, Entry 8). In both these reactions, there could be an increased potential to form ethyl iminium ion **2.5**. One potential explanation for these results is that iminium ion **2.5** may combine with magnesium salts to form intermediates like **2.4a** (Scheme 3.10), where the presence of an ethyl group allows the prospect of ethene elimination to produce phthalimide **1.75**. There is also the possibility that the iminium ion **2.5** plays the role of the hydride acceptor in the ethene elimination to generate ethylamide **2.12**, as in Scheme 3.10 below. Although ethylamide **2.12** has been previously reported as a side-product in the tetraethylation of phthalimide **1.75**,[140] we did not observe the formation of this product in any of the Grignard reactions undertaken. However, it is possible that trace amounts of ethylamide **2.12** formed in the reaction mixture may not be stable under these conditions, as the presence of the

hydrogen atom may allow degradation through various ring-opening and elimination reactions. The proposed mechanism for the reformation of phthalimide **1.75** prompted us to examine the isolated yield of re-formed phthalimide if the Grignard methylation reaction was started from ethylhydroxy amide **2.2**. The regeneration of trace amounts of phthalimide **1.75** (3%, Table 3.1, Entry 7) indicates that product **2.2** may go through a mechanism unlikely to involve the iminium ion **2.5**,[140] but may produce an iminium ion such as **3.8a** (Scheme 3.9) further along in the reaction pathway, and this species may facilitate the formation of higher yields of **1.82**.[140] Evidence for loss of the ethyl group in the form of ethene as part of the reaction process is given by the result that adducts containing methyl only, **3.1**, **3.2** and **3.3**, were formed during reactions starting with partially ethylated starting materials (Table 3.1, Entries 5, 7 and 8).

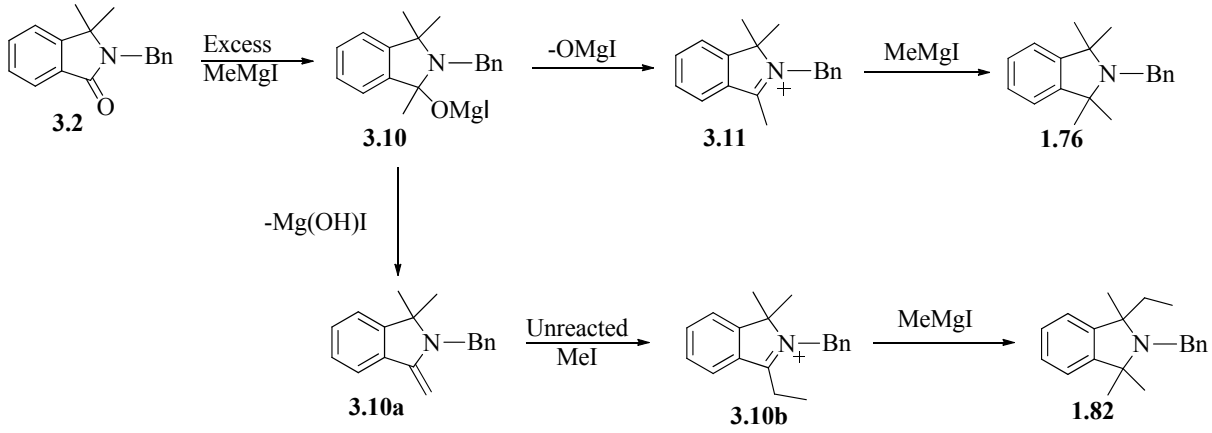


Scheme 3.10 One possible mechanism for the reformation of **1.75** from **2.2** & **2.5**.

3.2.3 Further reaction of 1,1-dimethyl amide side-product **3.2** under Grignard conditions.

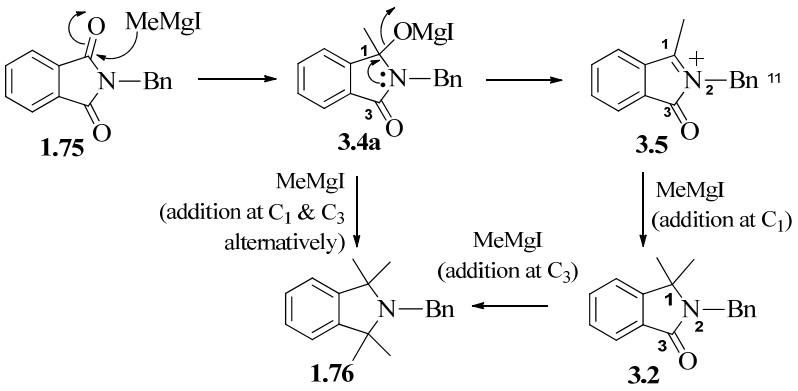
The other interesting side product formed in the reaction mixture was the dimethyl amide **3.2** (Scheme 3.11). This compound might be expected to be an intermediate on the pathway to the desired tetramethyl adduct **1.76**. Therefore, Grignard methylation was performed on dimethyl amide **3.2** in order to determine the

influence on the yield of **1.76** and the formation of side products. Subsequent treatment of product **3.2** with four equivalents of MeMgI in refluxing toluene for 72 hours gave products **1.76** (24%) and **1.82** (19%) along with trace amounts of unreacted starting material **3.2** (4%). The proposed mechanism of forming **1.76** and **1.82** from **3.2** has been shown in Scheme 3.11 below.

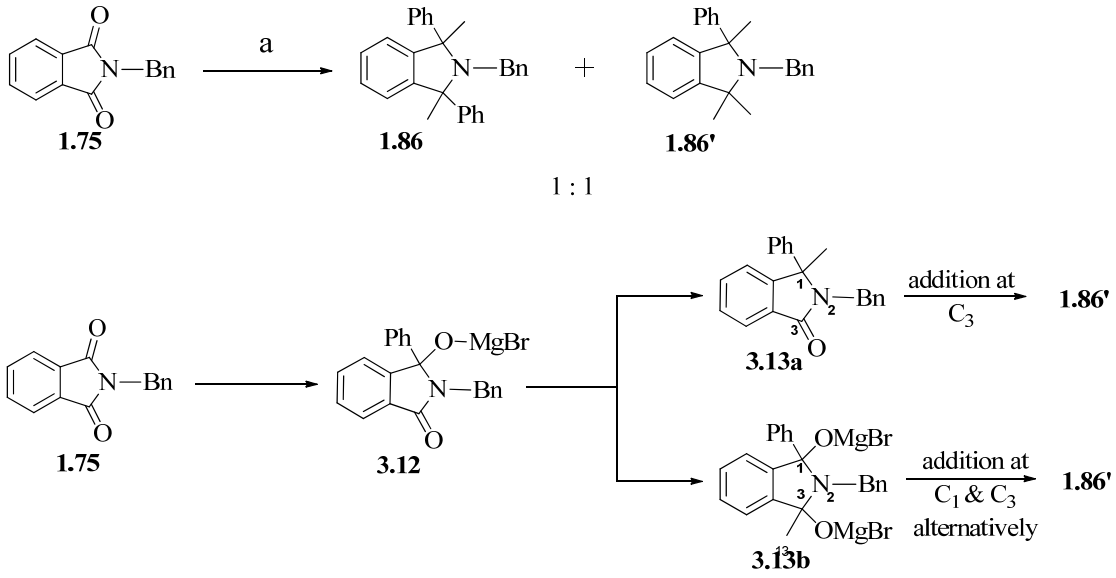


Scheme 3.11 Proposed mechanism for the formation of **1.76** and **1.82**, starting from dimethyl amide **3.2**.

Interestingly, dimethyl amide **3.2** was detected in the Grignard methylation of *N*-benzylphthalimide **1.75** (Table 3.1, Entry 1). This implies that the target tetramethyl adduct **1.76** could be formed *via* two pathways, either involving the alkoxy magnesium salt **3.4a** or *via* the methyl iminium intermediate **3.5** (Scheme 3.12). Braslau[108] did not observe the formation of dimethylamide **3.2** during their investigation of mixed phenyl and methyl Grignard treatments of the phthalimide **1.75**. These workers only detected the formation of 1,3-dimethyl-1,3-diphenylisoindoline **1.86**, where both initial methyl Grignard additions occurred on carbons 1 and 3.[108] They observed another product, 1,1,3-trimethyl-3-phenylisoindoline **1.86'** during the formation of **1.86** (1:1 ratio); however they didn't explain how the latter product is derived in the reaction.[108]



Scheme 3.12 Two pathways proposed for the formation of **1.76**, starting from phthalimide **1.75**.



Scheme 3.13 Two proposed pathways for the formation of **1.86'**, which was detected during Braslau's studies.

Reagents and conditions: (a) 1. PhMgBr (2.0 equiv.), toluene, rt-80 °C, 30 min.; 2. MeMgBr (4.0 equiv.), toluene, 111 °C, 12 h.[108]

The formation of side products including ethyltrimethyl adduct **1.82**, exocyclic alkene **3.1** and purple coloured intractable material during the exhaustive methyl Grignard treatment of phthalimide **1.75** is a likely cause for the low yield of the fully methylated adduct **1.76**. Though iminium ion **3.5** may convert to **1.76** over time, **3.5** can also react to give **3.1**, **3.2** and **3.3**. Conversion of dimethyl amide **3.2** to **1.76** is

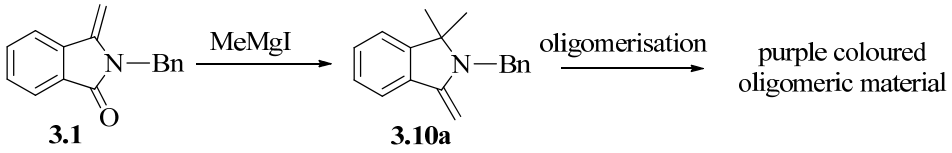
also associated with the formation of considerable amounts of ethyltrimethyl adduct **1.82** in the reaction mixture. The replacement of toluene with higher boiling xylenes (Table 3.1, Entry 3) gave similar final yields for **1.76** (~25%). Employing longer reaction times (Table 3.1, Entry 2) and the use of larger excesses of MeMgI[35,144] did not improve the yield of tetramethyl adduct **1.76**.

Table 3.4 Mass balance of the reaction when *N*-benzylphthalimide **1.75** was reacted with 6.0 equivalents of MeMgI at 110 °C for 4 h (Entry 1, Table 3.1).

Amount of **1.75** used as the starting material [mg]	Amount of the product recovered [mg]	Amount of the purple product recovered [mg]
500	400	~85

When the total mass balance of the reaction is analysed, less than 80% of the starting material can be accounted for in terms of alkylated species **1.76**, **1.82**, **3.1**, **3.2**, and **3.3**. The remainder of the phthalimide starting material is converted to a purple-coloured product (Table 3.4). In the previous chapter, the isolated yield of the tetraethyl analogue **1.94** was improved (up to 60% over two steps) by starting from the ethylhydroxy compound **2.2**, which provided a practical preparative scale improvement over the existing syntheses.[110,145] With this in mind, the partially methylated product **3.3** was subjected to methylation (Table 3.1, Entry 4). However, although the methylhydroxy amide **3.3** is expected to efficiently convert to the iminium ion **3.5** during the Grignard reaction, the yield of **1.76** remained modest (20%, Table 3.1, entry 4). This is due to competitive dehydration of **3.3** to give the exocyclic alkene **3.1**. Another prospect for the observed modest yield of **1.76** is that conversion of **3.3** into a purple-coloured product: more than 20% (mass balance) of the purple product was isolated from this reaction. Earlier Griffiths and co-workers hypothesised that oligomerisation of the enamine species **3.10a** may be responsible

for the formation of the purple coloured product during this Grignard reaction.[19] Enamine **3.10a** (Scheme 3.14) was not detected by us; however it may form from **3.1** during the reaction. Since enamide **3.1** derives from the dehydration of methylhydroxy amide **3.3**, this is a likely route for the formation of the purple-coloured oligomeric material.



3.1 **3.10a**

Scheme 3.14 Griffith's hypothesis of the formation of purple product.

Based on the results described in the previous chapter, conversion of the hydroxyl amide **3.3** into a molecule with a better leaving group could more readily lead to iminium ion intermediates that are involved further along the sequence that generates tetramethyl adduct **1.76**. They could therefore deliver the tetramethyl target **1.76** in a higher yield. Consequently, methylhydroxy amide **3.3** was modified with a methoxy leaving group by reacting with MeI in the presence of a base as described below.

3.2.4 Synthesis of 2-Benzyl-3-methyl-3-methoxyisoindolin-1-one (**3.4b**)

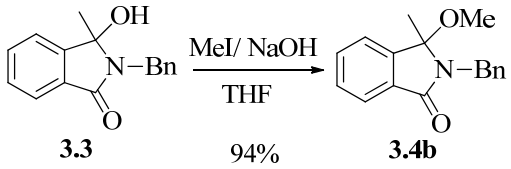
3.2.4.1 Background

The methylmethoxy amide **3.4b** has been synthesized previously by Tomooka and co-workers in 2006, starting from **3.3**,[141] but the experimental procedure for this synthesis or characterization of this compound were not reported.

3.2.4.2 Synthesis of **3.4b**

We previously synthesized the ethyl analogue of **3.4b** (Chapter 2) by reacting ethylhydroxy amide **2.2** with MeI in the presence of NaOH in THF. This reaction was optimized with 4 equivalents of MeI and NaOH to obtain a yield of 93% (Table

2.3, entry 4) for the ethyl analogue **2.3b**. Following a similar procedure gave the methoxymethyl amide **3.4b** in a yield of 94% with >95% purity by HPLC (Scheme 3.15).

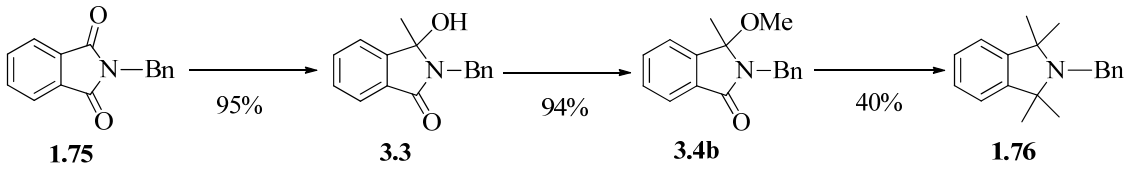


3.3 94% **3.4b**

Scheme 3.15 Synthesis of methylmethoxy amide **3.4b** starting from **3.3**.

3.2.4.3 Exploring the Grignard methylation using methylmethoxy amide **3.4b** as the starting material

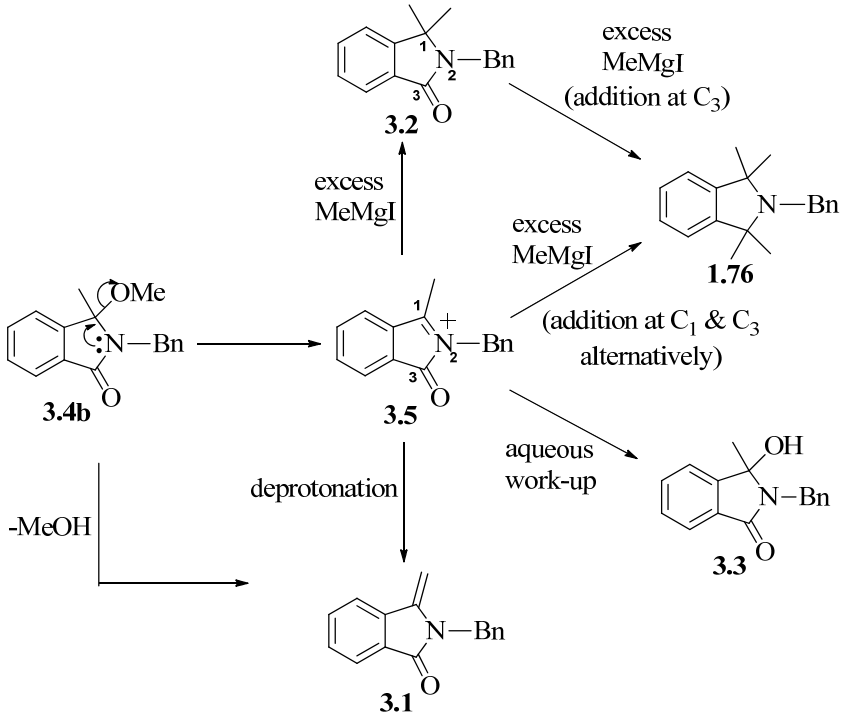
Subsequent treatment of **3.4b** with six equivalents of MeMgI in refluxing toluene for 72 hours gave the tetramethyl adduct **1.76** in 36% yield (Table 3.1, Entry 6). However, the overall yield of **1.76** calculated over three steps (32%) is still lower than the best yield observed (37%) for tetramethyl adduct **1.76** starting from phthalimide **1.75**. Notably higher amounts of the dimethyl amide **3.2** and hydroxymethyl amide **3.3** were formed during this reaction. Since both **3.2** and **3.3** can be converted to **1.76**, the isolated yield of **1.76** should improve further if greater excesses of MeMgI are employed. With this in mind, six equivalents of MeMgI were refluxed with **3.4b** for 72 hours and then an additional six equivalents of MeMgI were added to the above reaction, followed by further 72 hour reflux, with the expectation that **3.2** and **3.5** in the reaction mixture would be converted to **1.76** by reaction with excess MeMgI. However, this approach gave only slightly improved yields for **1.76** (40%) even though there was a notable decrease in the yields of **3.2** (7%) and **3.3** (2%).



Overall yield of **1.76** over three steps = 36%

Scheme 3.16 Alternative 3-step route for the synthesis of **1.76**.

From the mass balance of this reaction, it was determined that about 40% of the starting material **3.4b** is transformed into side products, including the purple-coloured material. Though the yield of tetraethyl product **1.94** was improved by the better leaving group strategy,[140] the yield of tetramethyl adduct **1.76** was not improved in the same manner which is believed to be because of the formation of the purple-coloured product.



Scheme 3.17 One proposed pathway for the formation of **1.76, 3.1, 3.2** and **3.3** from methylmethoxy amide **3.4b**.

The purple-coloured material appears to be a complex oligomer that is not readily characterised by chromatographic and spectroscopic techniques. Considerable efforts

were employed to purify and identify this compound, which were largely unsuccessful. However, a few structural features were revealed which give some indications as to the nature of this product.

3.2.5 Investigating the formation of oligomeric purple-colored material

The formation of purple-coloured material was observed during the tetramethylation of several different starting materials, including phthalimide **1.75**, enamide **3.1**, methylhydroxy amide **3.3** and methylmethoxy amide **3.4b**. Each purple-coloured oligomeric product, regardless of the substrate used, gave a similar TLC retention factor (using 2-methylbutanol:AcOH 8:3 as eluent, which was kindly suggested by Dr. Kye Masters), containing one major spot along with a faster running minor spot. Several solvent combinations were employed to dissolve/precipitate the oligomeric product, including dichloromethane (DCM) & cold toluene, DCM & cold diethyl ether, DCM & cold hexane and DCM & cold cyclohexane. In all cases, the purple material was dissolved in a minimum amount of warm DCM and then filtered. The filtrate was then added dropwise to the cold solvent, which induced precipitation, and the suspension was kept for 1-2 days to allow the solid to settle to the bottom of the flask. The supernatant was discarded and the precipitate was collected by centrifugation. Since the precipitation process was best with DCM & cold cyclohexane, the purple material was iteratively precipitated (ten times) and the final sample collected and analysed by TLC (using 2-methylbutanol : acetic acid, 8:3 as eluent). Nonetheless, the TLC plate showed an unchanged character.

The material was subsequently passed through a silica column using 2-methylbutanol:acetic acid 8:3 as eluent. This gave faster running component as a solid. Analysis using ^{1}H-NMR spectroscopy, HPLC and TLC indicated that this compound was *N*-benzylphthalimide **1.75**. The eluent was then modified (2-

methylbutanol:AcOH, 2:1, 4:3); however, the purple material did not elute from the column. The polarity of the eluent was further increased to 1:1 and another 10% MeOH was added, but the major component was still not observed in the collected fractions. Increased insolubility upon purification or irreversible binding to the silica may be the reason why chromatography was not successful in purifying the purple material.

Purification of the purple product was then attempted using reversed phase medium pressure liquid chromatography (MPLC), with THF/H_2O 9:1 as eluent. It was not possible to use greater than 15% H_2O in the eluent due to solubility problems. The same minor component isolated as per silica column chromatography was again isolated and was shown to be *N*-benzylphthalimide **1.75**. Therefore, the THF portion in the eluent was increased to 100% and the reversed phase column was washed for another one hour. When the collected fraction was concentrated and analysed by TLC, the expected major component was not observed.

Size exclusion chromatography (SEC) was next employed as a means to gain insight into the structure of the purple oligomer by considering its mass. The SEC chromatogram (Figure 3.1) generated for the precipitated (from DCM and cyclohexane) purple material consisted of several broad peaks (as a series) within the range of 25-30 minutes. From this result, it was speculated that the purple product may be composed of several oligomers of less than 1000 Da (polystyrene-equivalent molecular weight). Matrix-assisted laser desorption ionoization time-of-flight mass spectrometry (MALDI-ToF-MS) was undertaken on the precipitated purple material (see Experimental section for sample preparation) and the obtained mass spectrum showed a poorly defined complex mixture of masses

with values ranging from 600-1000 (m/z). The potential structures of the purple coloured oligomeric mixture were determined based on this mass range.

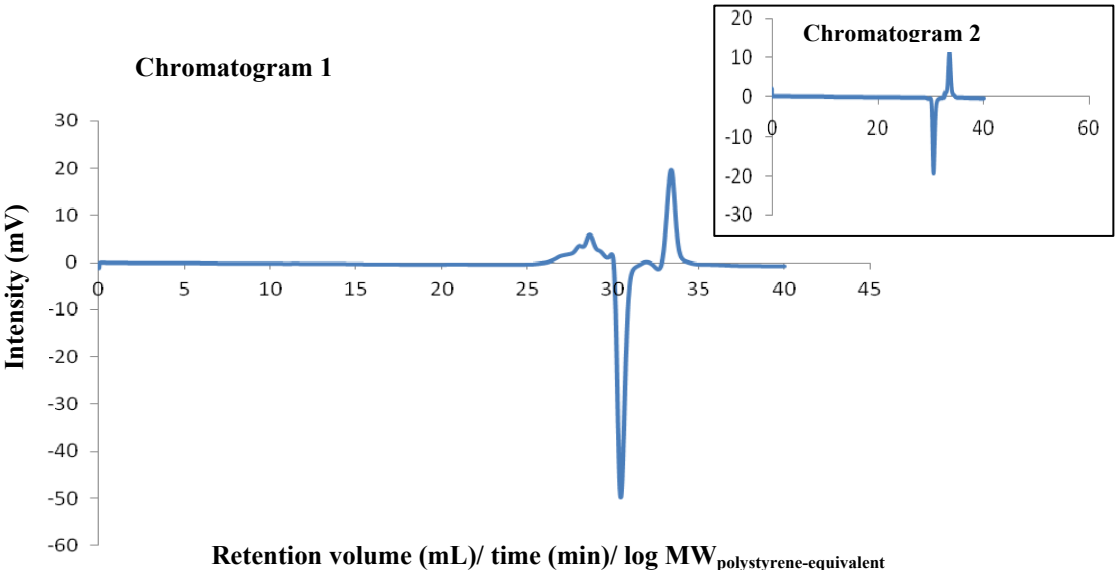


Figure 3.1 Size Exclusion Chromatograms of the purple-coloured oligomer (Chromatogram 1, THF) and the GPC system blank (Chromatogram 2, THF).

The ^{1}H-NMR spectrum (Figure 3.2) of the precipitated purple-coloured oligomeric material displayed signals in the aromatic region and the alkyl region. There were no signals that could be attributed to a significant amount of benzylic -CH_2- groups in the purple coloured material. The integration of the aromatic region compared to the alkyl region indicated that there was a low degree of unsaturation in the product. However, one region (1.5 – 2.2 ppm) of the ^{1}H-NMR spectrum was broad and therefore it was difficult to interpret whether this broad region represented H atoms of the molecule or the broad region signifies impurities overlapping with the actual peaks of the molecule. The ^{13}C-NMR spectrum (Figure 3.2) showed a very weak peak in the carbonyl region (δ 167.6ppm), however it was predicted that the previously detected *N*-benzylphthalimide **1.75** (having a carbonyl peak at δ 168 ppm) in the purple oligomeric material could have attributed to this carbonyl group in the ^{13}C-NMR spectrum.

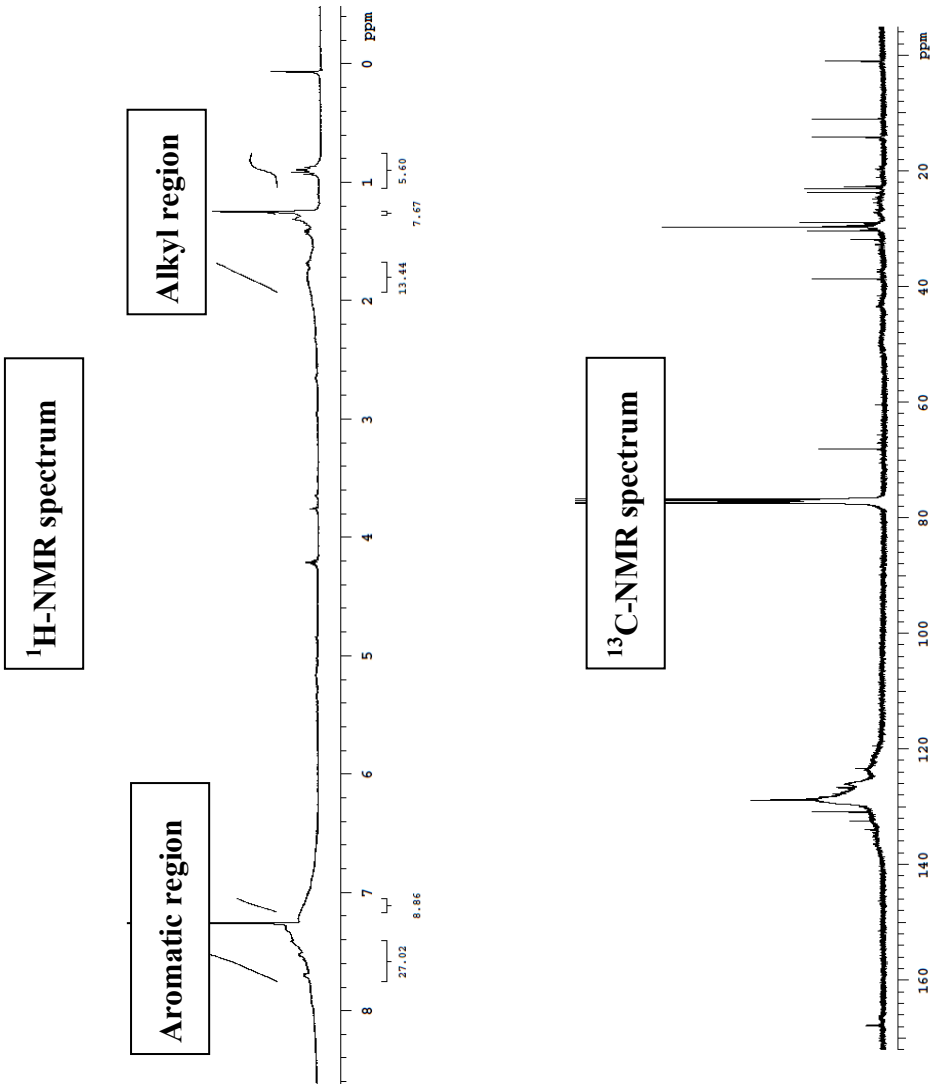


Figure 3.2 ^{1}H-NMR & ^{13}C-NMR spectra of the precipitated purple material ($CDCl_3$)

This prompted us to analyse the ^{1}H-NMR spectrum (Figure 3.2) of the purple material for the presence of any benzyl -CH_2- groups as the benzylic -CH_2- peak of *N*-benzylphthalimide **1.75** appears at δ 4.84 ppm. Absence of such a benzyl -CH_2- peak in the ^{1}H-NMR spectrum and the absence of any strong carbonyl group signal by IR spectroscopy showed that *N*-benzylphthalimide **1.75** in the purple material might have been removed by the series of precipitations. Therefore, the peak appearing at 167.6 ppm may be attributable to an imine carbon, which characteristically appears within a range of

δ 160-169 ppm depending on the environment.[146] Two deuterated solvents, $CDCl_3$ and $(CD_3)_2CO$, were used for the analysis of the purple oligomeric material by NMR spectroscopy; however $CDCl_3$ was preferable to $(CD_3)_2CO$ due to improved solubility.

The other characteristic feature, the strong purple colour of the oligomeric material, may imply a considerable degree of conjugation and interaction with non-bonding electron pairs. Analysis of the purple oligomer by Ultraviolet-Visible spectrometry showed a broad peak in the region of 500-650 nm (Figure 3.3), indicative of extended conjugation in the structure.

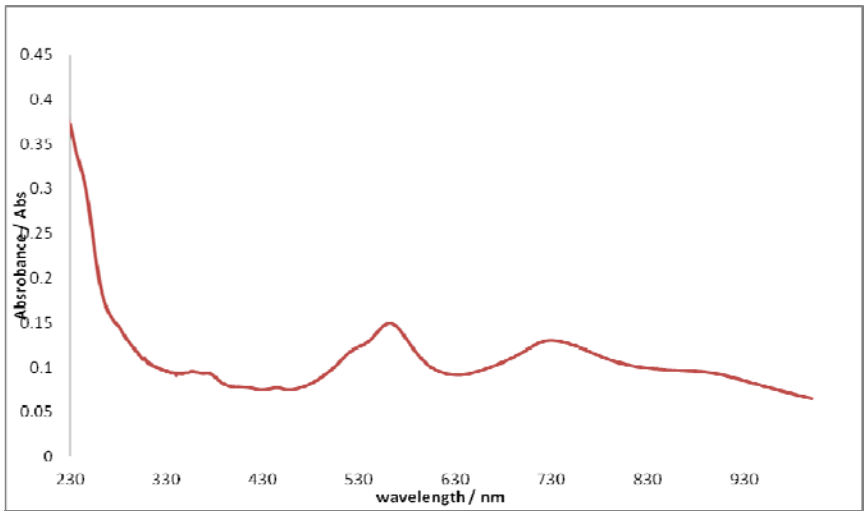
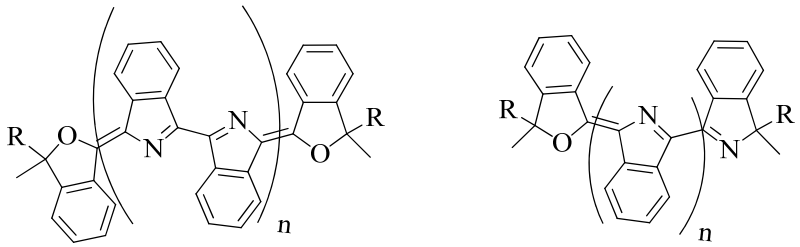


Figure 3.3 UV-Visible spectrum of the precipitated purple material (DCM).

The formation of the oligomeric material is dependant on generation of alkene **3.1**. Such a purple coloured by-product does not form in ethyl Grignard reactions where exocyclic alkenes are not detected. The purple-coloured oligomer was generated in much higher yield when alkene **3.1** was used as the substrate instead of *N*-benzylphthalimide **1.75**. The mass balance of the reaction showed ~40% of substrate **3.1** was converted to the purple oligomer during the Grignard methylation. From these findings, it seems likely that the oligomeric mixture consists of a mixture of

components with each having a number of isoindolic rings condensed into a conjugated system. Some possible structures that could be present in the purple oligomeric mixture are given in Figure 3.4 below.

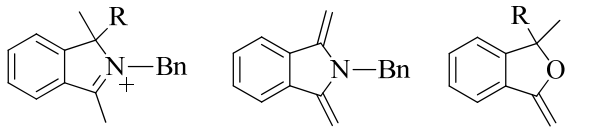


n = 1-3 R = Et or Me n = 1-6

3.14 **3.15**

Figure 3.4 Some proposed structures that could be present in the purple coloured oligomeric mixture.

The formation of the purple oligomer demonstrates one of the reasons why the yield of tetramethyl adduct **1.76** is limited. The generation of purple by-product was most evident when the Grignard methylation was performed on **3.1** instead of *N*-benzylphthalimide **1.75**. The purple oligomer was also generated when the Grignard was performed on methylhydroxy amide **3.3** and methylmethoxy amide **3.4b**. Interestingly, **3.1** is a byproduct when Grignard tetramethylation was undertaken on substrates such as *N*-benzylphthalimide **1.75** (Entry 2, Table 3.1), methylhydroxy amide **3.3** (Entry 4, Table 3.1) and methylmethoxy amide **3.4b** (Entry 6, Table 3.1). Therefore, it is reasonable to assume that exocyclic alkene **3.1** plays a significant role in the formation of the unwanted purple-coloured byproduct.



3.8c **3.16** **3.17**

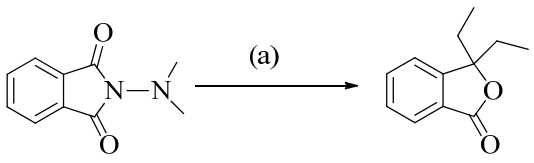
R = Et : **3.8b**

R = Me : **3.11**

R = Et or Me

Figure 3.5 Possible monomer units that could co-oligomerize to form **3.14** & **3.15**

The ethyl (**3.8b**) and methyl (**3.11**) versions of **3.8c** (Figure 3.5) were earlier introduced as intermediates during the formation of ethyltrimethyl adduct **1.82**, starting from **3.1** (Scheme 3.8) and the formation of tetramethyl adduct **1.76**, starting from 1,1-dimethyl amide adduct **3.2** (Scheme 3.11) respectively. Structure **3.16** could be derived from the reaction of 1 equivalent of MeMgI with the carbonyl group of enamide **3.1** followed by elimination of Mg(OH)I.[142] The formation of structures like **3.17** in the Grignard reaction mixture seem unlikely, however a similar reaction was earlier noted by Scheffler and co-workers[129] during ethylation of **3.18** with excess EtMgBr (Scheme 3.18).



3.18 **3.19**

Scheme 3.18 A side-product (**3.19**) isolated during the Grignard ethylation of **3.18**[129]

Reagents and conditions: (a) EtMgBr, 90-100 °C.[129]

3.3 CONCLUSIONS

Clearly the Grignard reaction using MeMgI at high temperatures gives rise to a number of structurally modified derivatives of *N*-benzlphthalimide **1.75**. The range of potential reactions and complexity of both the possible pathways and the reaction

intermediates lead to the formation of a partially defined purple oligomeric mixture that is characteristic of this reaction. The experiments described in this chapter showcase some of the important intermediates and by-products generated in the exhaustive methylation of *N*-benzylphthalimide **1.75** using MeMgI. The insights gained in this study may prove useful in the synthesis of other isoindolinic nitroxides, such as the heteroaromatic systems described in the following chapter.

3.4 MANUSCRIPT OF THE WORK RELATED TO CHAPTER 3

(To be submitted to *Australian Journal of Chemistry*)

Further Insights into the Exhaustive Grignard Tetramethylation of *N*-Benzylphthalimide

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Abstract

Exhaustive Grignard tetramethylation of *N*-benzylphthalimide is referred to as the critical step in the synthesis of valuable isoindoline nitroxide TMIO, as it is found to be yield limiting. Analysis of this reaction through a different reaction work-up demonstrated the formation of some hitherto unreported side products which do not appear to be intermediates on the pathway to form the 2-benzyl-1,1,3,3-tetramethylisoindoline. It was however revealed that the yield was significantly limited due to the formation of a purple coloured complex mixture which is characteristic to this route. It was also found that a range of potential reactions and complications of the side-products lead to the formation of this complex purple

Chapter 4: Synthesis of Pyridine Annulated Heterocyclic Nitroxides with an Improved Yield

4.1 BACKGROUND

Isoindoline nitroxides possess some advantages over other classes of nitroxides. Their wide-spread application has been hampered by the challenges involved with their large scale synthesis, many of which have been discussed in previous chapters. Heteroaromatic analogues of isoindoline nitroxides have received even less attention, even though the presence of the heteroatom can convey some important properties. For instance, heterocyclic nitroxides such as the imidazolidine nitroxides display preferential sensitivity towards oxidative processes, and have been used as EPR probes in biomedicine and related fields to monitor oxidative stress and reactive radical species in biological systems.[119] Heterocyclic nitroxides can also be used as pH sensitive spin probes. The best examples of such nitroxides are imidazoline and imidazolidine-based nitroxides[118a] which show changes in the EPR spectrum as the pH of the aqueous medium is varied.[118b] In addition, heterocyclic nitroxides are also important as contrast enhancing agents[8] for magnetic resonance imaging (MRI) applications as well as molecular units[120a,120b] in the synthesis of molecular magnetic materials. There can, however, be some disadvantages associated with monocyclic nitroxides containing heteroatoms. For instance, the electron-withdrawing effect of the heteroatom in the ring can destabilise the positive charge of the resulting oxo-ammonium cation.[117] The heteroatom can also facilitate ring-opening reactions[76,119]

in monocyclic nitroxides, and thereby promote degradation (Scheme 1.29, Chapter 1).

These issues are circumvented if a heteroaromatic ring is fused to the monocyclic nitroxide skeleton. In addition, the presence of a fused pyridine-like heterocycle could impart some advantages such as rigidity and resistance towards ring-opening reactions,[76] good σ-donor capabilities as a monodentate ligand,[121] and resistance towards the alteration of functions of biomolecules when they act as spin labels.[123] Thus the latter part of this thesis focuses on applications of the synthetic understanding developed in earlier Chapters to generate a novel heteroaromatic isoindoline nitroxide analogue.

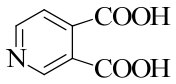
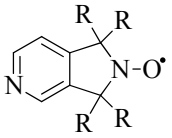
Although some methods have previously been described to synthesise pyrroline nitroxide-annulated pyridine systems, they were produced in very poor yields *via* complex multistep sequences. Hideg and co-workers reported a method of synthesising a pyridine-fused paramagnetic nitroxide system using a hetero Diels-Alder cyclisation of symmetric paramagnetic diene **1.62** with *N*-(butoxycarbonylmethylene)-*p*-toluenesulfonamide to give 6-butoxycarbonyl-1,3,4,5,6,7-hexahydro-1,1,3,3-tetramethyl-5-(*p*-toluenesulfonyl)-2*H*-pyrrolo[3,4-*c*]pyridine-2-yloxyl radical[32] **1.102a**, which was further hydrolysed, aromatised, and esterified to generate nitroxide **1.102**[123] (Scheme 1.30, Chapter 1). The diene starting material **1.62** had to be synthesised from commercially available nitroxide **1.67** in six steps,[89,93] and the overall synthetic yield of **1.102** starting from compound **1.67** was only 4%. Another approach to the synthesis of pyrroline nitroxide-annulated pyridine systems has been the Sonogashira coupling of aldehyde-containing nitroxide **1.68** with phenylacetylene to give **1.105a**, of which oxime **1.105b** spontaneously cyclised to the paramagnetic nitroxide derivative **1.105** upon heating[124a] (Scheme 1.32,

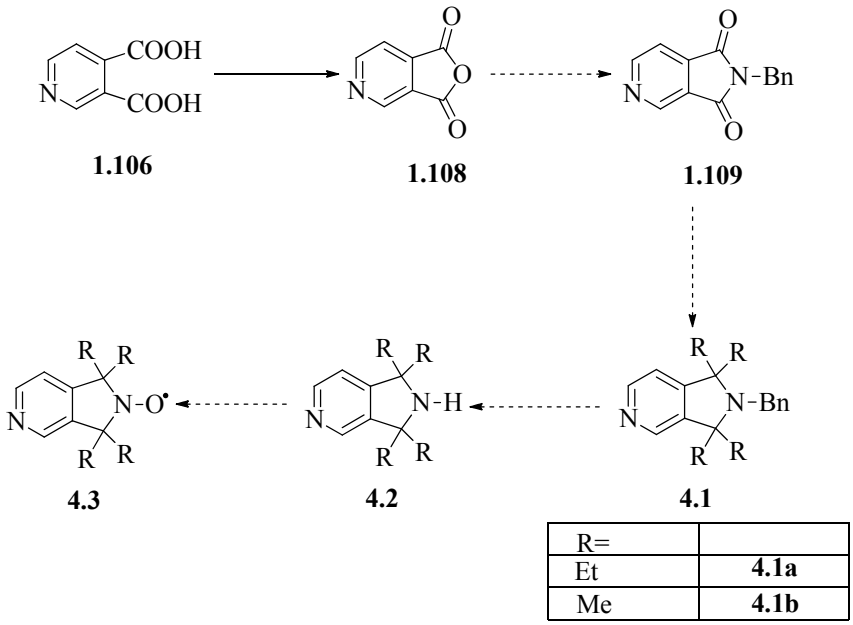
Chapter 1). Since aldehyde-containing nitroxide **1.68** was not commercially available, it was synthesised from TEMPONE (**1.19**, Scheme 1.19, Chapter 1) *via* 4 steps with an overall yield of 18%.[101,102] The overall synthetic yield of substituted pyridine-annulated nitroxide **1.105** starting from commercially available **1.19** was 10%. A third approach to the synthesis of pyridine-fused nitroxides has been described by Roggero and co-workers using Cobalt catalysed cyclysation reactions.[124b] In this method, Cobalt(0) catalyses the cyclocotrimerization of tetramethyldipropargylamine **1.71** with acetonitrile to give 2,3-dihydro-1,1,3,3,6-pentamethyl-1*H*-pyrrolo[3,4-*c*]pyridine **1.106**[124b] (Scheme 1.33, chapter 1), which can be oxidized to the corresponding paramagnetic nitroxide derivative. There are some draw-backs associated with this method as the tetramethyl amine **1.106** was not converted into the corresponding paramagnetic nitroxide and the synthesis of **1.106** was confirmed only by Gas chromatography (98%).[124b] To date, the best quantitative synthetic yield of pyridine fused pyrroline nitroxide was 10% via the second approach described above (Scheme 1.32, chapter 1).[123]

Since tetraalkylation of *N*-benzylphthalimide **1.75** provides reasonable yields and limited reaction steps for the formation of TEIO (**1.13**) and TMIO (**1.15**) compared to other methods, the synthesis of pyridine-annulated tetraalkyl nitroxides through the Grignard alkylation of *N*-benzylcinchomeronic imide **1.109** followed by hydrogenation and oxidation is an attractive approach. Herein we report a short (5 step) and convenient approach for the synthesis of unsubstituted pyridine-annulated pyrroline nitroxides with much improved yields.

4.2 OUTLINE OF THE PROPOSED SYNTHETIC STRATEGY

Table 4.1 Structures of the starting material and the synthetic target of the project

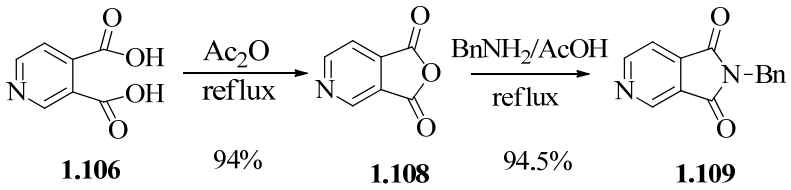
Starting material	Target
 **1.106**	 **4.3**



Scheme 4.1 Proposed reaction pathway to synthesize **4.3**

4.3 RESULTS AND DISCUSSION

The synthesis of novel pyridine-annulated nitroxide **4.3** began with the dehydration of commercially available pyridine-3,4-dicarboxylic acid **1.106**. Pyridine-3,4-dicarboxylic anhydride **1.108** was succefully synthesised starting from **1.106** in good yield *via* refluxing in acetic anhydride.



Scheme 4.2 Synthesis of **1.109** from commercially available **1.106**.[147a]

Following the method of Mayor and Wentrup,[147b] the diacid was refluxed in acetic anhydride for 30 minutes followed by sublimation under reduced pressure to give the desired anhydride in 94% yield.

4.3.1 Synthesis of *N*-Benzylcinchomeronic imide **1.109**

Conversion of anhydride **1.108** to imide **1.109** was undertaken following a method analogous to that used for the synthesis of *N*-benzylphthalimide **1.75**. Sublimed anhydride **1.108** was refluxed for 2 hours with 2.0 equivalents of benzylamine in acetic acid to give the title compound in a yield of 94.5% (Scheme 4.2).

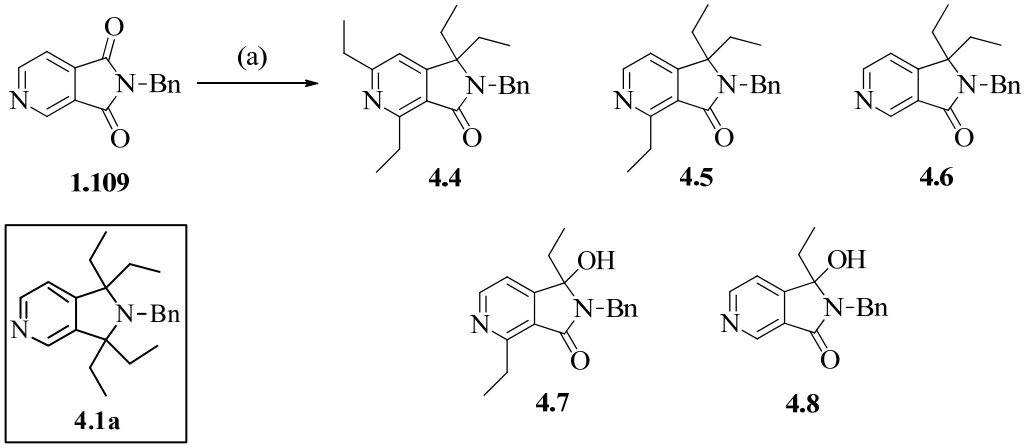
4.3.2 Tetraalkylation of *N*-Benzylcinchomeronic imide **1.109**.

Tetraalkylation of imide **1.109** commenced with ethylation, as it has been previously shown for benzyl-protected phthalimide that alkylation with an ethyl as opposed to a methyl Grignard reagent leads to an increase in yield. Grignard reagent EtMgI was chosen over the chlorides and bromides due to higher reactivity.

4.3.2.1 Attempted tetraethylation of imide **1.109**

Tetraethylation of imide **1.109** was attempted following a method analogous to that used for the synthesis of tetraethylisoindoline adduct **1.94**, starting from phthalimide **1.75**. Imide **1.109** was reacted with six equivalents of EtMgI in toluene at reflux for five hours followed by quenching with saturated ammonium chloride. The organic layer was separated and the volatiles removed. The aqueous ammonium chloride layer was treated with sodium carbonate solution as it was expected that the slightly acidic nature of ammonium chloride would protonate the pyridine ring. The

precipitated sodium chloride was filtered off and the aqueous layer was then extracted with chloroform. The mixture was analysed by TLC (hexane:ethyl acetate, 3:2) and the five spots observed on the TLC plate were isolated by column chromatography. These five components were identified by NMR and IR spectroscopy and Mass spectrometry (Scheme 4.3).



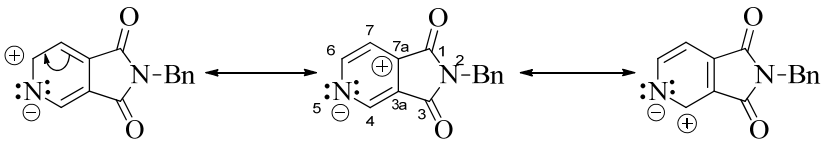
Scheme 4.3 Products obtained during the reaction of **1.109** with excess EtMgI.

Reagents and conditions: (a) EtMgI (6.0 equiv.), toluene, 110 °C, 5 h; **4.4** (2%), **4.5** (6%), **4.6** (9%), **4.7** (17%), **4.8** (37%).

These five products were confirmed in the HPLC chromatogram by running the isolated compounds with the same conditions (eluent: MeOH/H_2O 65:35). Further analysis by ramping to 90% methanol/ 10% water for 10 minutes, then holding for 50 minutes showed no discernable peaks assignable to the desired tetraethyl adduct **4.1a**. Therefore, it was concluded that the most non-polar products and the least non-polar product formed during this reaction were **4.4** and **4.8** respectively. This indicated that tetraethyl adduct **4.1a**, which could be expected to be the least polar product, has not been formed in the reaction under the given experimental conditions.

The five products shown in Scheme 4.3 should have another regio isomer as the starting imide **1.109** contains two carbonyl groups. Therefore some of the products

(shown in Scheme 4.3) were analyzed by two dimensional NMR to find out which carbonyl group had not reacted with the EtMgI. Although IR and ^{13}C-NMR spectroscopy indicated that one carbonyl group remained present in all the five compounds isolated, further analysis was required to find which carbonyl had not reacted in each case. The HMBC (Heteronuclear Multiple-Bond Correlation) spectrum showed that the H atom attached to carbon 4 in structure **4.8** (Figure 4.1) correlates with the carbonyl carbon 3 (by a correlation spot marked as number 1) in the HMBC spectrum (Figure 4.1). This enabled us to rule out structure **4.8a** from the two possible regio-isomers.



1.109a **1.109b** **1.109c**

Scheme 4.4 Resonance structures of the imide **1.109**.

One possible explanation for the puzzling non-reactivity of one carbonyl group of **1.109** towards the ethylation could be described by considering the resonance formation of imide **1.109** (Scheme 4.4). The positive charge at C_{7a} on the resonance structure of **1.109b** may make the adjacent carbonyl carbon (C1) more electropositive compared to the carbonyl carbon (C3) neighbouring on the opposite side. Therefore, the nucleophilic ethylating agent would probably react with the more electropositive carbonyl carbon, readily giving a series of products from **4.4** to **4.8**.

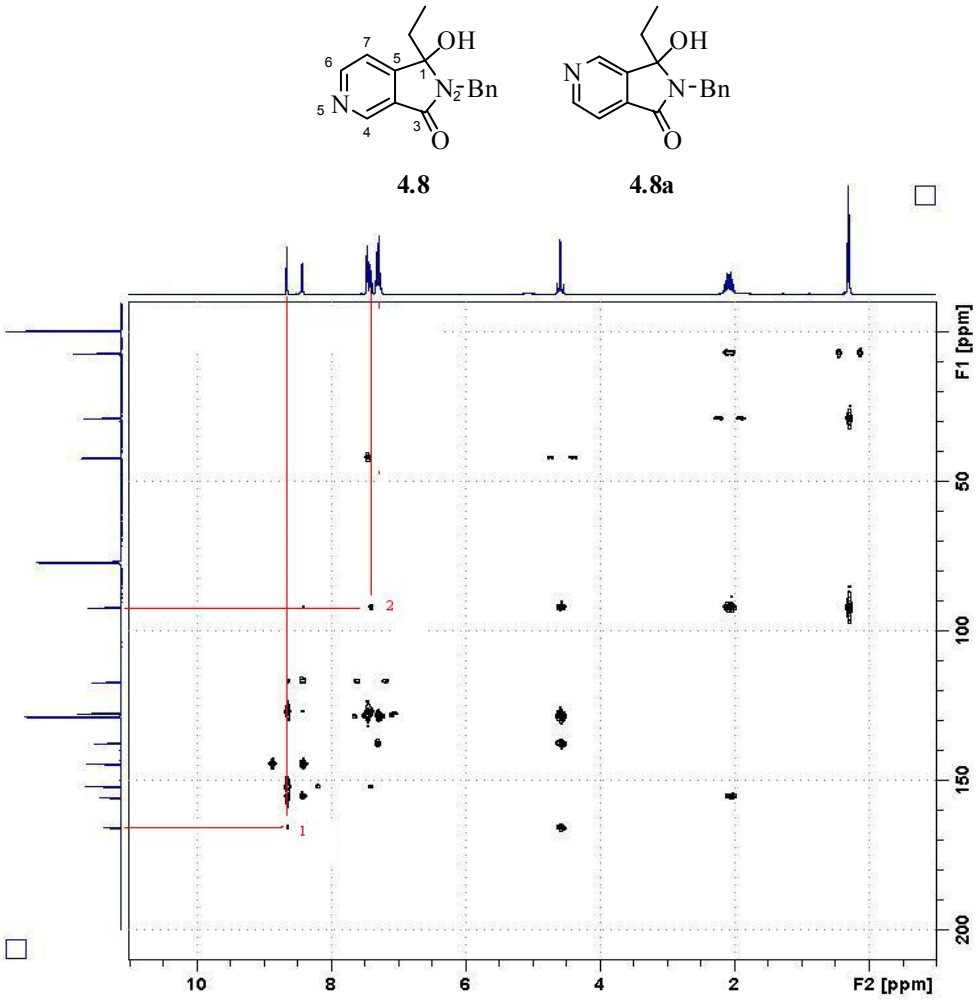
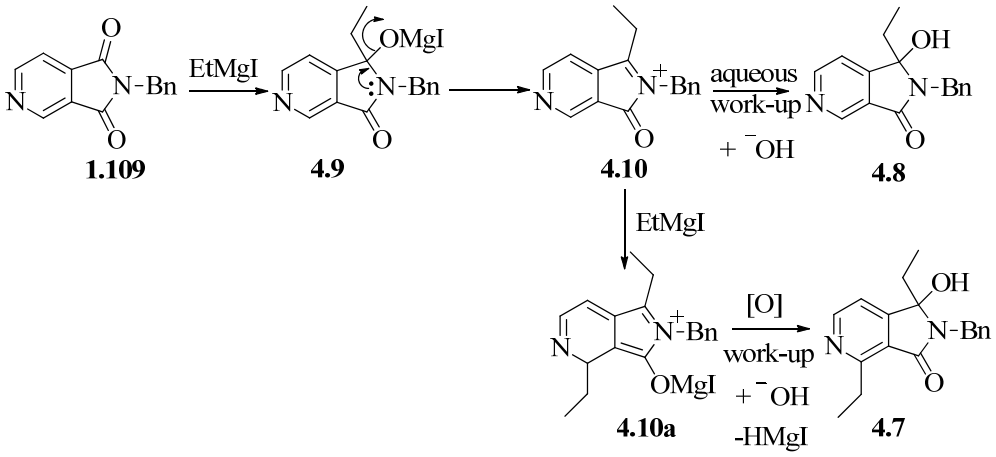


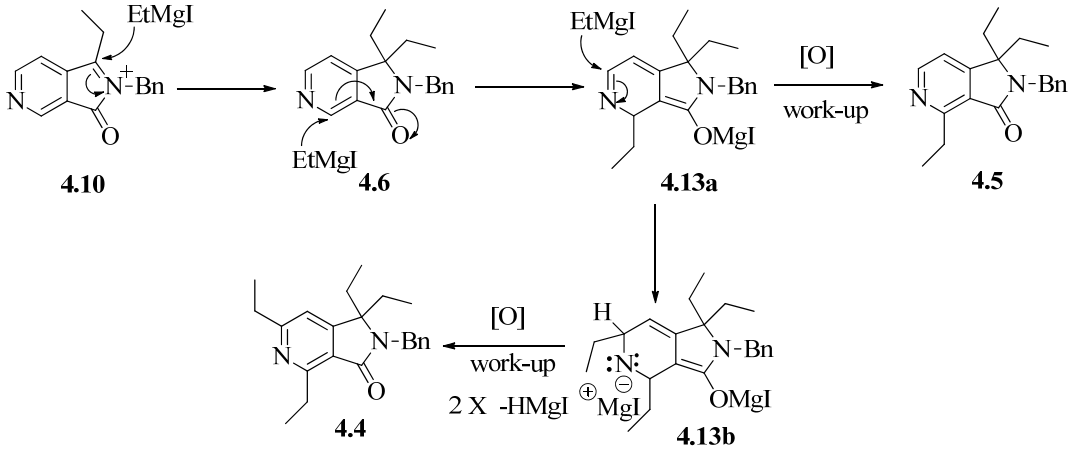
Figure 4.1 HMBC spectrum of the product **4.8**.

According to the structures of the five products (Scheme 4.3), it is clear why the desired tetraethyl adduct **4.1a** was not formed during the ethylation of imide **1.109**. As described in previous work,[140] it was concluded that 1,1-diethyl amide derivatives **4.4**, **4.5** and **4.6** may not appear as intermediates on the pathway to the desired tetraethyl adduct **4.1a**. Ethylhydroxy amide **4.8** may be derived from iminium ion **4.10** as a result of the aqueous work-up. It seems that iminium ion **4.10** could undergo ring ethylation to produce a structure like **4.10a**, which shows resistance to further alkylation.[58] This intermediate **4.10a** would later re-aromatise and re-oxidise to **4.7** during the aqueous work-up[58] (Scheme 4.5).



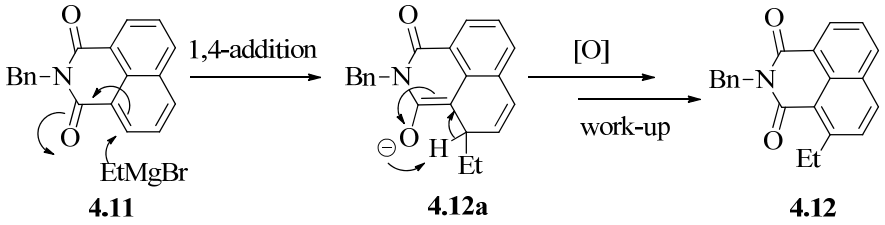
Scheme 4.5 One proposed pathway of forming **4.7** and **4.8**.

Diethyl amide **4.6** could arise from iminium ion **4.10** by reacting with one equivalent of EtMgI. This diethyl amide **4.6** would then undergo further ring ethylation to produce **4.4** and **4.5**. It is likely that the enone system of structure **4.6** may act as a Michael acceptor leading to a 1,4-addition at the fourth position of the pyridine ring to give rise to **4.13a** (Scheme 4.6). The pyridine ring of **4.13a** would then re-aromatize and re-oxidize during the work-up to yield **4.5**. A similar reaction to this was suggested for the formation of **4.7** as well (Scheme 4.5). This type of a reaction has been previously observed during an attempt to tetraethylate *N*-benzyl-1,8-naphthalimide **4.11** using EtMgBr in toluene at reflux (Scheme 4.7).[58]



Scheme 4.6 One possible pathway of forming **4.4**, **4.5** and **4.6**.

The researchers did not observe any of the desired tetraethyl adduct in the reaction, but observed **4.12** as a major product which could be formed *via* a long-lived intermediate such as **4.12a** which later re-aromatized and re-oxidized during the work-up to give **4.12**.[58]



4.11 **4.12a** **4.12**

Scheme 4.7 Proposed mechanism detailing the prevention of reactivity of phthalimide **4.11** towards further ethylation.[58]

However it is possible that **4.5** could have been derived not only from **4.6** (Scheme 4.6) but also from **4.10a** as well. To rule out this suggestion, a series of ethyl Grignard reactions on imide **1.109** were undertaken with several different reaction times (Table 4.2) and the reaction mixture of each reaction was analysed by HPLC. These data indicated that **4.6** appeared in the reaction mixture initially and then **4.5** and **4.4** were formed with time. Presumably diethyl amide **4.6** may act as a precursor for both ring-alkylated diethyl amide derivatives **4.4** and **4.5**. Since the intermediate **4.10a** could be formed within the first 15 minutes along with diethyl amide **4.6** (Entry 5, Table 4.2), it is posible that **4.5** could be derived from both **4.10a** and **4.6**. Similarly **4.4** would be derived either from **4.6** (*via* **4.13a**) or **4.10a** or from both. According to the established literature, it is well know that pyridine systems undergo nucleophilic substitution relatively easily due to the presence of nitrogen atom in the ring.[131] (This could be the reason why ring alkylation was not observed with the isoindoline system).

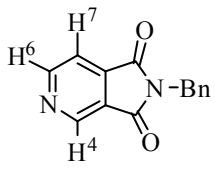
Table 4.2 Products observed with pyridine ring ethylation and carbonyl ethylation.

Entry	Equiv. EtMgI	Reaction temp[°C]	Reaction time [h]	Products observed in the reaction mixture				
				4.4	**4.5**	**4.6**	**4.7**	**4.8**
1	4.0	110	5	●	●	●	●	●
2	4.0	110	3.5	●	●	●	●	●
3	4.0	110	1.5	-	●	●	●	●
4	4.0	110	1	-	-	●	●	●
5	4.0	110	1/4	-	-	●	●	●

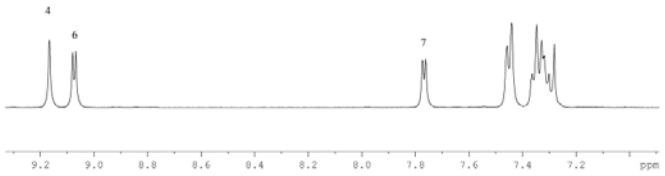
● product observed / - product not observed.

The location of the ethyl groups on the pyridine ring of **4.4** and **4.5** were identified by comparing the aromatic regions (^{1}H-NMR spectra) of imide **1.109**, diethylamide derivatives **4.4** and **4.5**. No singlet corresponding to H^4 is visible in the ^{1}H-NMR of **4.5** due to the substituted ethyl group at C4 (compare with imide **1.109**, which shows the C4 proton at δ 9.15 ppm). In the ^{1}H-NMR of **4.4**, a singlet appears due to the presence of one H atom in the pyridine ring. However the δ (ppm) value of this singlet indicates that this H atom is attached to a carbon which is not adjacent to the N atom of the pyridine ring of **4.4** (Figure 4.2). The observed differences in the chemical shifts (δ) of H^6 and H^7 in the two ^{1}H-NMR spectra of compounds **1.109** and **4.5** (Figure 4.2) demonstrate the changes in the chemical environment (of H^6 and H^7) due to the addition of ethyl groups at the carbonyl carbon of **4.5**.

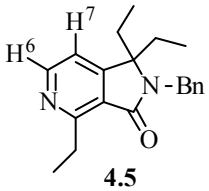
Spectrum 1



1.109

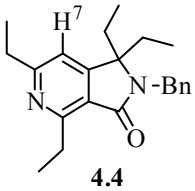


Spectrum 2



4.5

Spectrum 3



4.4

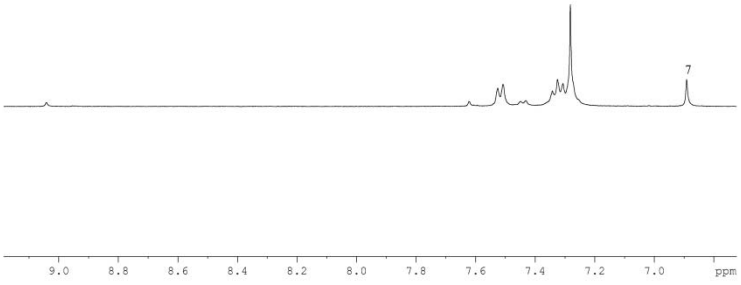


Figure 4.2 Inspection of the aromatic regions of the ^{1}H-NMR spectra of imide **1.109** and substituted diethyl amide derivatives **4.4** and **4.5**.

The two pyridine ring H's of **4.5** (that is H^6 and H^7) could be doublets (in a ^{1}H-NMR spectrum) with two possible regio isomers, **4.5** and **4.5'** (Figure 4.3). The regio-isomer **4.5'** was however ruled out using NOESY (Nuclear Overhauser Effect Spectroscopy). This indicated the correlation (marked as spot Y) between methyl H's of carbon 1b and H^7 through space (Figure 4.3). As has been suggested previously, both **4.4** and **4.5** could be derived from diethyl amide **4.6**, therefore it was concluded that all the three structures, **4.4**, **4.5** and **4.6** would have one pattern of regio-isomerism.

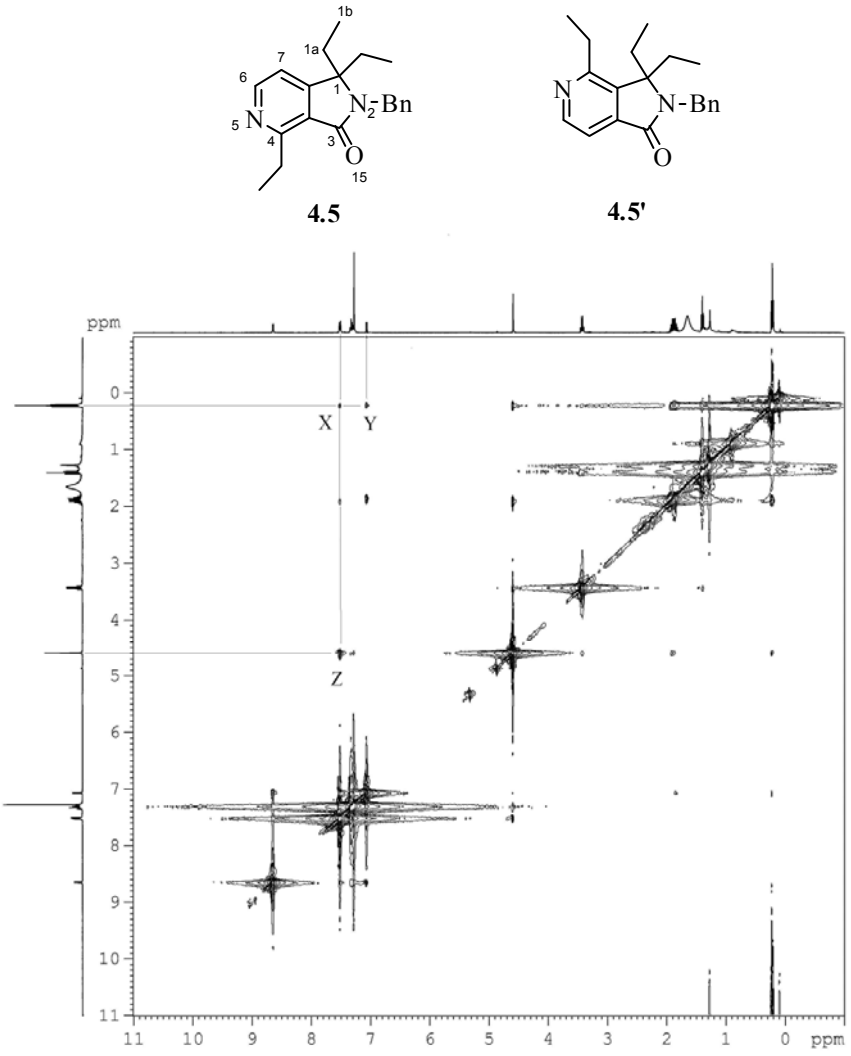


Figure 4.3 NOESY spectrum of the product **4.5**.

After analysing all the results, it was concluded that substrate **1.109** was not driven to the desired tetraethyl adduct **4.1a** due to the formation of side products such as **4.4**, **4.5**, **4.6** and **4.7** which do not undergo further ethylation. If ring ethylation of **4.10** could be eliminated, it could possibly be converted to target **4.1a**. Therefore it was decided to investigate some Grignard ethylation reactions on imide **1.109** by optimising the experimental conditions to discover under which experimental conditions ring ethylation could be eliminated.

Since the formation of ring-ethylated hydroxyl amide **4.7** was observed in the reaction mixture after merely 15 minutes of refluxing (Entry 5, Table 4.2), imide **1.109** was reacted with EtMgI at a decreased temperature (Table 4.3). The HPLC analysis of each reaction mixture indicated that **4.7** was formed even at 60 °C. (Previously it has been observed that ring ethylation can occur at low temperatures[58]).

Table 4.3 Products observed in the reaction mixture of Grignard addition to **1.109** with decreasing the reaction temperature

Entry	Equiv. EtMgI	Reaction temp[°C]	Reaction time [h]	Products observed in the reaction mixture				
				4.4	**4.5**	**4.6**	**4.7**	**4.8**
1	4.0	110	72	●	●	●	●	●
2	4.0	80	72	-	-	-	●	●
3	4.0	60	72	-	-	-	●	●

● product observed / - product not observed

Decreasing the number of equivalents of EtMgI eliminated the ring ethylation (Entry 3, Table 4.4), but the amount of EtMgI remaining in the reaction mixture was not sufficient for further ethylation of the intermediates. An alternative route for the

synthesis of desired **4.1a** was designed staring from **4.8**, as the isoindoline analogous reaction of this[140] gave significantly higher yield for the tetraethyl adduct **1.94** *via* a path which was unlikely to involve an iminium ion such as **4.10**. (The work-up of the reaction mixtures shown in Table 4.3 and 4.4 was carried out by quenching the Grignard reaction mixture first with aqueous NH_4Cl, separating the organic phase, and then combining the organic phase with the chloroform extraction of the aqueous NH_4Cl layer).

Table 4.4 Products observed in the reaction mixture of Grignard addition to **1.109** with decreasing the equiv. of EtMgI.

Entry	Equiv. EtMgI	Reaction temp[°C]	Reaction time [h]	Products observed in the reaction mixture				
				4.4	**4.5**	**4.7**	**4.8**	**4.9**
1	6.0	110	3.5	●	●	●	●	●
2	4.0	110	3.5	●	●	●	●	●
3	2.0	110	3.5	-	-	-	-	●

● product observed / - product not observed

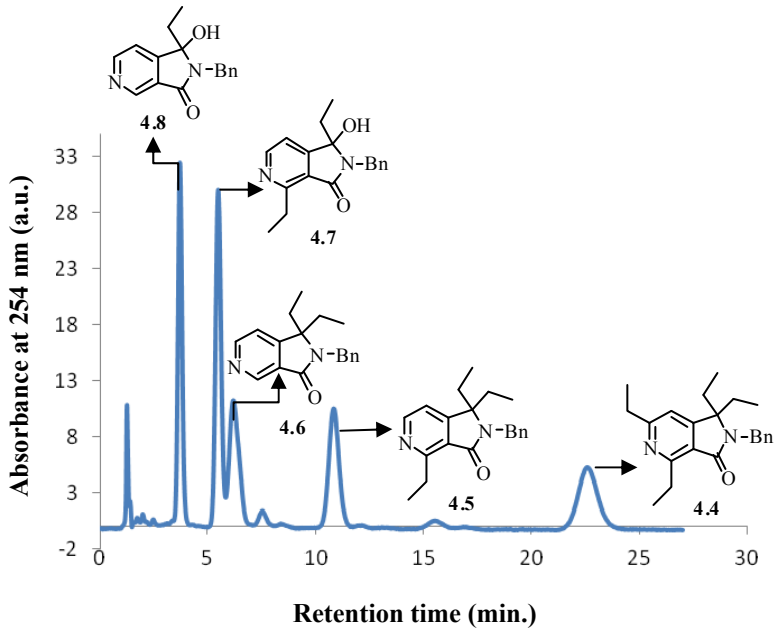


Figure 4.4 HPLC chromatogram of the reaction mixture when imide **1.109** was reacted with excess EtMgI at 110 °C for 72 hours (Entry 1, Table 4.3).

(**Elution method:** MeOH/H_2O 65:35 for 27 minutes)

4.3.2.1.1 Synthesis of 2-Benzyl-1-ethyl-1-hydroxy-1*H*-pyrrolo[3,4-*c*]pyridin-3(2*H*)-one **4.8**

Synthesis of ethylhydroxy amide **4.8** from imide **1.109** was performed following a method analogous to that used for the synthesis of **2.2** starting from phthalimide **1.75**. Reacting imide **1.109** with 2.5 equivalents of EtMgI at room temperature for 2 hours, quenching the mixture with aqueous NH4Cl, followed by extracting the aqueous layer with chloroform and evaporating the combined organc layers, gave a chromatogram as shown in Figure 4.5, upon analysis by HPLC. The title ethylhydroxyl amide **4.8** (regio-isomerism was confirmed by HMBC, Figure 4.1) was isolated by column chromatography (hexane/ethylacetate, 1:1) with a yield of 43%.

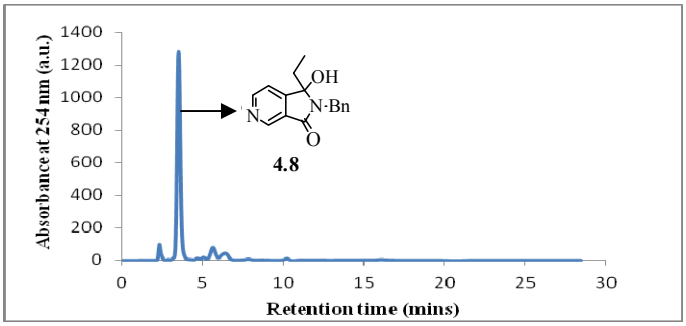
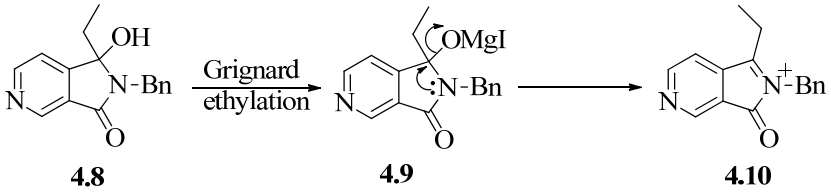


Figure 4.5 HPLC chromatogram given when **1.109** was reacted with 2.5 equivalents of EtMgI at room temperature for 2 hours.

(**Elution method**: see Figure 4.4)

When ethylhydroxy amide **4.8** was treated with excess EtMgI at toluene reflux for 5 hours, it showed the formation of previous side-products, **4.4**, **4.5**, **4.6** and **4.7** in the reaction mixture by HPLC. It seems that ring ethylation blocks the formation of the

expected O-Mg-O bridging complex (such as **2.16**),[140] so that the reaction would proceed through iminium intermediate **4.10** (Scheme 4.8), generating the dead-end products. Further experiments were not attempted with the Grignard ethylation of imide **1.109** due to the formation of these products, which do not react further under the Grignard conditions.

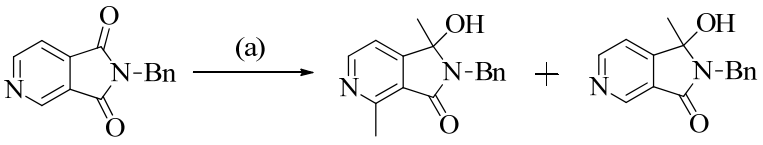


4.8 **4.9** **4.10**

Scheme 4.8 One possible pathway of forming iminium ion **4.10** starting from **4.8** during Grignard ethylation.

4.3.2.2 Tetramethylation of *N*-benzylcinchomeronic imide **1.109**.

Tetramethylation of imide **1.109** to synthesize 2-benzyl-1,1,3,3-tetramethyl-1,3-dihydro-1*H*- pyrrolo[3,4-*c*]pyridine (**4.1b**) was carried out similar to the procedure of tetramethylation of *N*-benzylphthalimide **1.75**.[35] Starting imide **1.109** was reacted with six equivalents of MeMgI in refluxing toluene for 3.5 hours. The reaction mixture was quenched with saturated NH_4Cl and the organic layer was separated. The aqueous ammonium chloride layer was extracted to chloroform, combined and evoporated. Analysis of the combined organic layers by HPLC (eluent: MeOH/H2O, 65:35 for 15 minutes, ramp to MeOH/H_2O 90:10 for 10 minutes and held for another 25 minutes) indicated that the reaction mixture consisted of two products. Isolation of the two products by column chromatography (hexane/ethylacetate 4:1) and identification by NMR spectroscopy and Mass spectrometry showed methylhydroxyamide **4.16** and the ring-substituted product **4.15** (Scheme 4.9).



1.109 **4.15** **4.16**

Scheme 4.9 Products obtained during the reaction of **1.109** with excess MeMgI.

Reagents and conditions: (a) MeMgI (6.0 equiv.), Toluene, 110 ˚C, 3.5 h

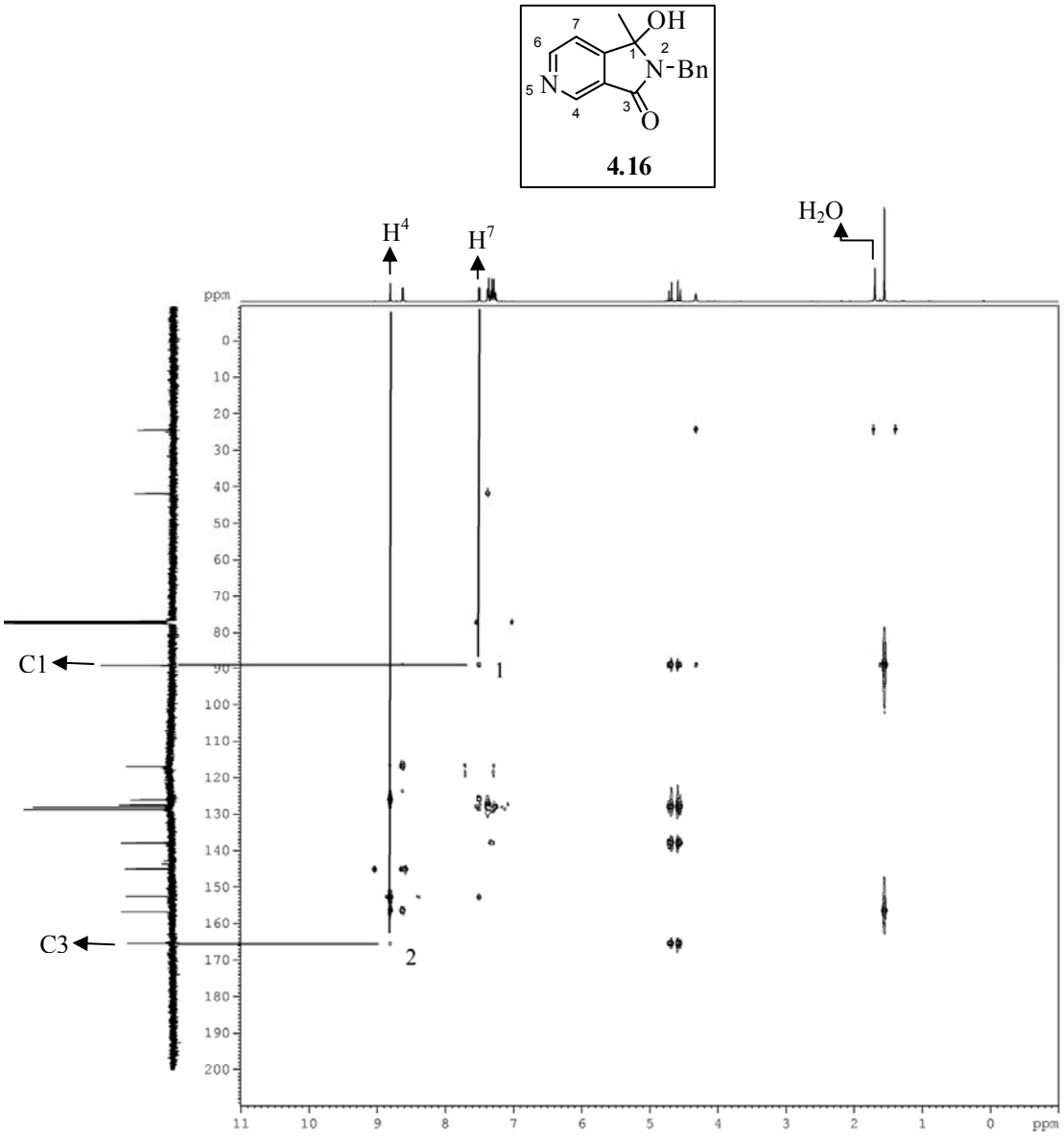


Figure 4.6 HMBC spectrum of methylhydroxy amide **4.16**

The regio-isomers shown in Scheme 4.9 were identified by 2D-NMR spectroscopy (HMBC). The HMBC spectrum of **4.16** (Figure 4.6) shows the correlations between

C1 and the H atom attached to C7 in structure **4.16** (marked as 1). Furthermore, it shows the correlations between the carbonyl C3 and the H atom attached to C4 of **4.16** (spot marked as 2). The position of methyl substitution in the pyridine ring of **4.15** was identified by the ^{1}H-NMR and HMBC spectroscopy; in the ^{1}H-NMR spectrum of **4.15**, there is a doublet appearing at δ 8.4-8.5 ppm. Structure **4.15** and **4.15a** are the only possible structural arrangements which could give a downfield doublet for a pyridine aromatic H (H^{6} in **4.15** or **4.15a**). But it is not possible to form structure **4.15a**, due to the deactivation of the adjacent carbonyl group once the ring methylation occurs at C4 of **4.15a** (see later for explanation). If regio-isomer **4.15b** is considered, the ^{1}H-NMR spectrum should indicate two down-fielded singlets (δ ~8-9 ppm) as both H^{6} and H^{4} are adjacent to the N atom of the pyridine ring (see spectrum 1, Figure 4.2). Therefore, both **4.15a** and **4.15b** can be ruled out.

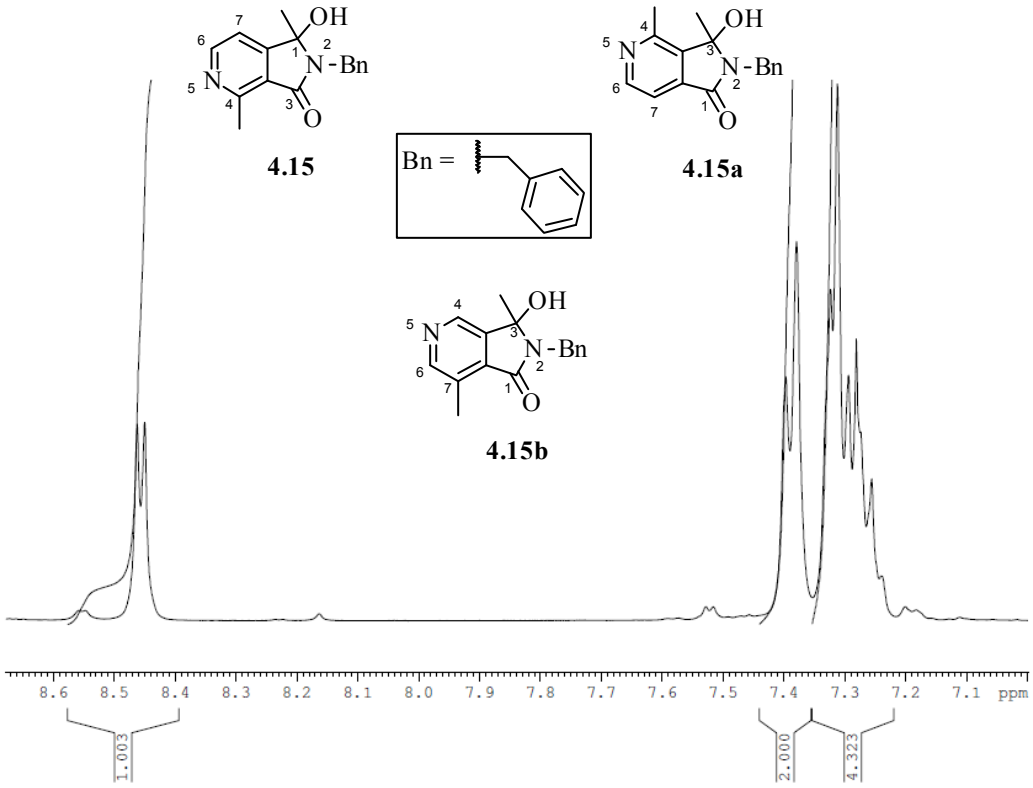


Figure 4.7 Inspecting the intigrations of the aromatic region of the ^{1}H-NMR spectrum of **4.15**.

The HMBC spectrum of **4.15** (Figure 4.8) also shows correlations (spot marked as 1) between the proton at C7 and C1 in structure **4.15**. However, it was difficult to identify H^7 of **4.15** in the ^{1}H-NMR as the integrations of the aromatic region show that H^7 overlaps with the five benzyl aromatic H atoms (Figure 4.7). Any of the aromatic H's of the benzyl group would not show correlations with C1 of **4.15** as all these benzylic aromatic H's are more than five bonds away. This proves that structure **4.15** is the regio-isomer formed in the reaction mixture.

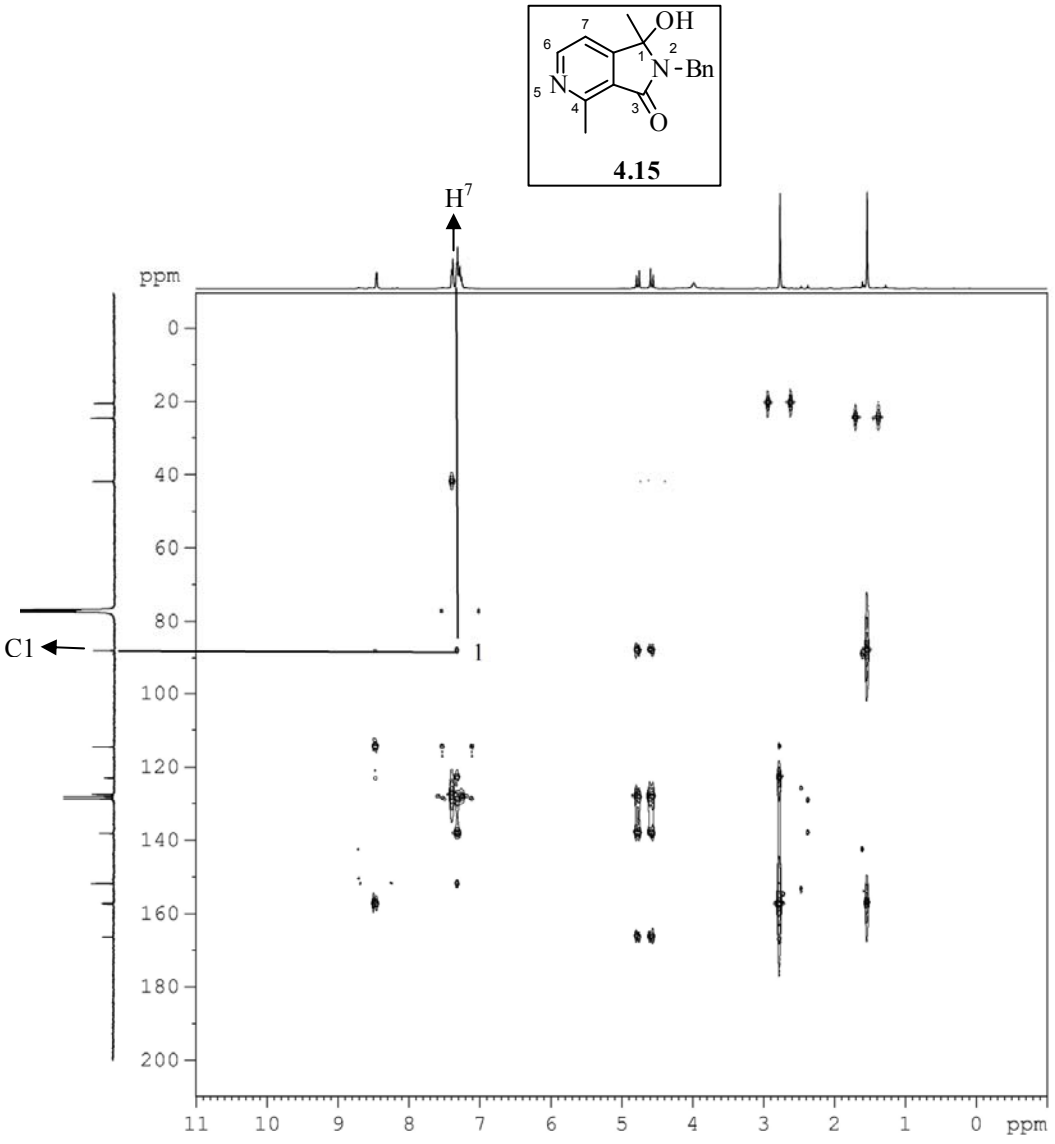


Figure 4.8 HMBC spectrum of the product **4.15**.

As the initial aim of this project was to synthesize tetramethyl adduct **4.1b**, the methylation of imide **1.109** was undertaken by extending the reflux time for five hours. The HPLC of the reaction mixture (Figure 4.9) showed appearance of two relatively non-polar products along with previously identified **4.16** and **4.15**. The two new products were isolated by column chromatography (hexane:ethyl acetate, 1:3) and identified by NMR and mass spectrometry as the tetramethyl adduct **4.1b** and ethyltrimethyl adduct **4.14a** (or **4.14b**, Figure 4.9). It seems likely that long refluxing time (3.5 h to 5 h) has influenced the other three methylations to occur on methylhydroxy amide **4.16** to form desired tetramethyl adduct **4.1b**. (The corresponding ethyltrimethyl adduct **1.82** was isolated during the tetramethylation of phthalimide **1.75** as well).[35]

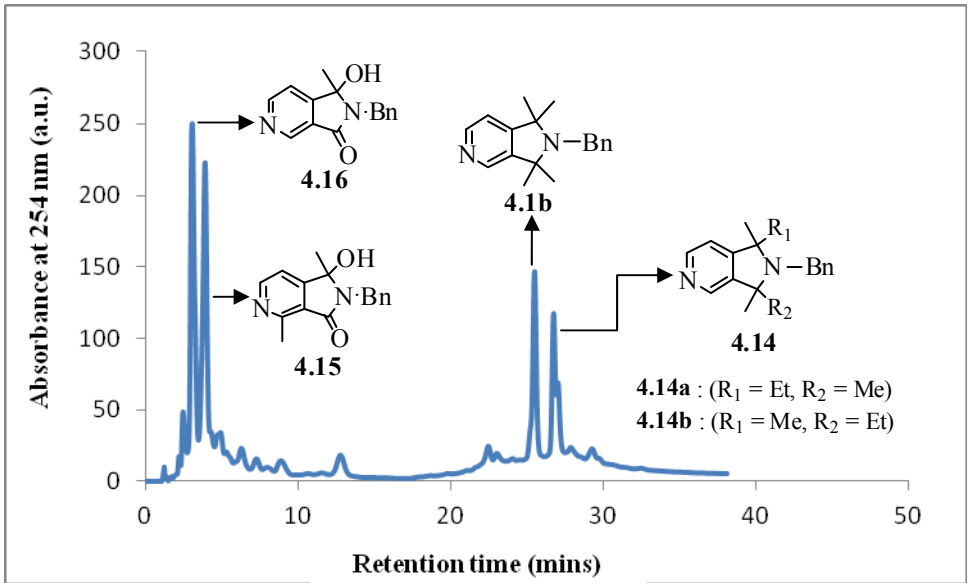


Figure 4.9 HPLC chromatogram of the reaction mixture shown in Scheme 4.10 (**Elution method:** MeOH/H_2O 65:35 for 15 minutes, ramp to 90:10 for 10 minutes, held for 15 minutes)

Comparison of the ^{1}H-NMR of ethyltrimethyl pyridine adduct **4.14a** (or **4.14b**) and isoindoline analogue **1.82** indicated similar peak patterns (Figure 4.10), and interestingly the methylene H signal of the benzyl group was split to a doublet (due

to the adjacent chiral centre) in both ^{1}H-NMR spectra, which is a characteristic feature of this structure. Both ^{1}H-NMR spectra show a triplet in the region of δ 0.5-0.7 ppm, which is due to the methyl portion of the ethyl group, and three singlets in the region of δ 1.25-1.45 ppm due to the presence of three methyl groups. Further, there are two multiplets appearing in the region of δ 1.30-1.70 ppm due to the two methylene H's of the ethyl group. In addition, the aromatic region of the ^{1}H-NMR spectrum of **4.14a** (or **4.14b**) shows two doublets and a singlet (each of the three peaks integrated for 1H; see appendix for intigrations: Figure A22) due to the three H atoms of the pyridine ring. This implies that this structure does not contain any substituted ethyl groups on the ring. This comparison and the mass spectroscopy (m/z = 280) finally confirmed the structure as **4.14a** (or **4.14b**).

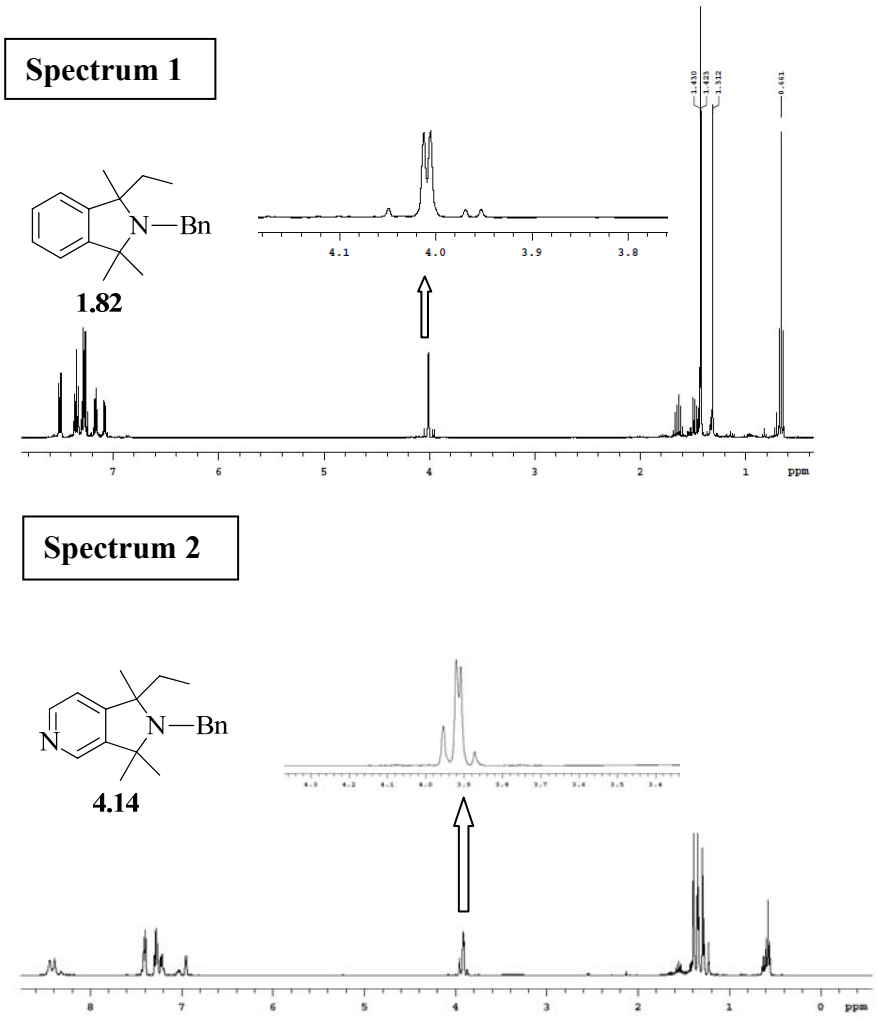
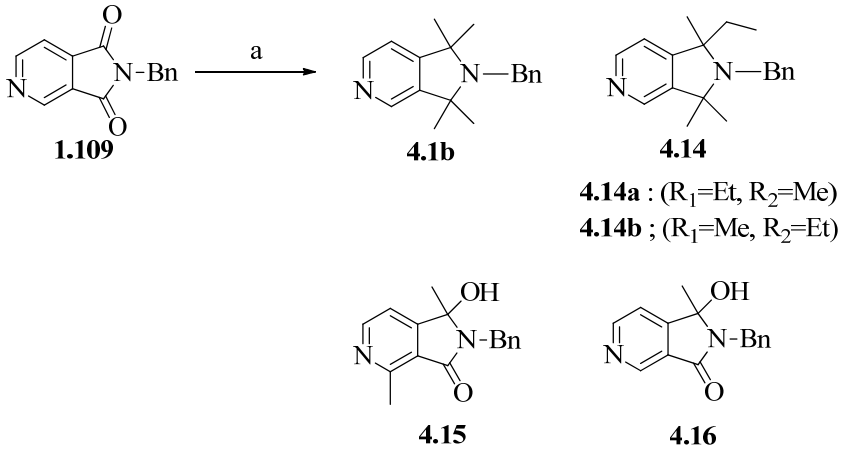


Figure 4.10 Benzylic peak splitting of **1.82** (Spectrum 1) and **4.14** (Spectrum 2).

Both ^{1}H-NMR and HPLC analysis of this product (**4.14a** or **4.14b**) demonstrated the presence of some tetramethyl adduct **4.1b** (~27%), which could not be separated either by column chromatography or recrystallization. The exact regio-isomer (**4.14a** or **4.14b**) of this ethyltrimethyl adduct was difficult to assume by 2D-NMR due to the problems encountered in purifying the product (see later).



Scheme 4.10 Products obtained during the refluxing of imide **1.109** with excess MeMgI.

Reagents and conditions: (a) MeMgI (6.0 equiv.), toluene, 110 °C, 5 h.

4.3.2.2.1 Optimizing the yield of tetramethyl adduct **4.1b** by modifying the experimental conditions

In order to optimize the yield of **4.1b**, a series of reactions was carried out (Table 4.5) by changing the refluxing time of the reaction, followed by HPLC analysis. HPLC allowed the direct analysis of the reaction mixture so that modification of the reaction conditions could be exploited to optimise the yield of the desired tetramethyl adduct **4.1b**. The product ratios of the four products, **4.16**, **4.17**, **4.14a** (**4.14b**) and **4.1b**, of each reaction, were determined as before, by considering the area under the curve of the chromatogram, so a HPLC yield represents the area of one peak compared to all the other peaks in the chromatogram. Comparison of the relative

HPLC yields of entry 1 and 2 (Table 4.5) indicates that ring-substituted methylhydroxy amide **4.15**, ethyltrimethyl adduct **4.14** and tetramethyl adduct **4.1b** are all likely to be derived from further methylation of methylhydroxy amide **4.16**.

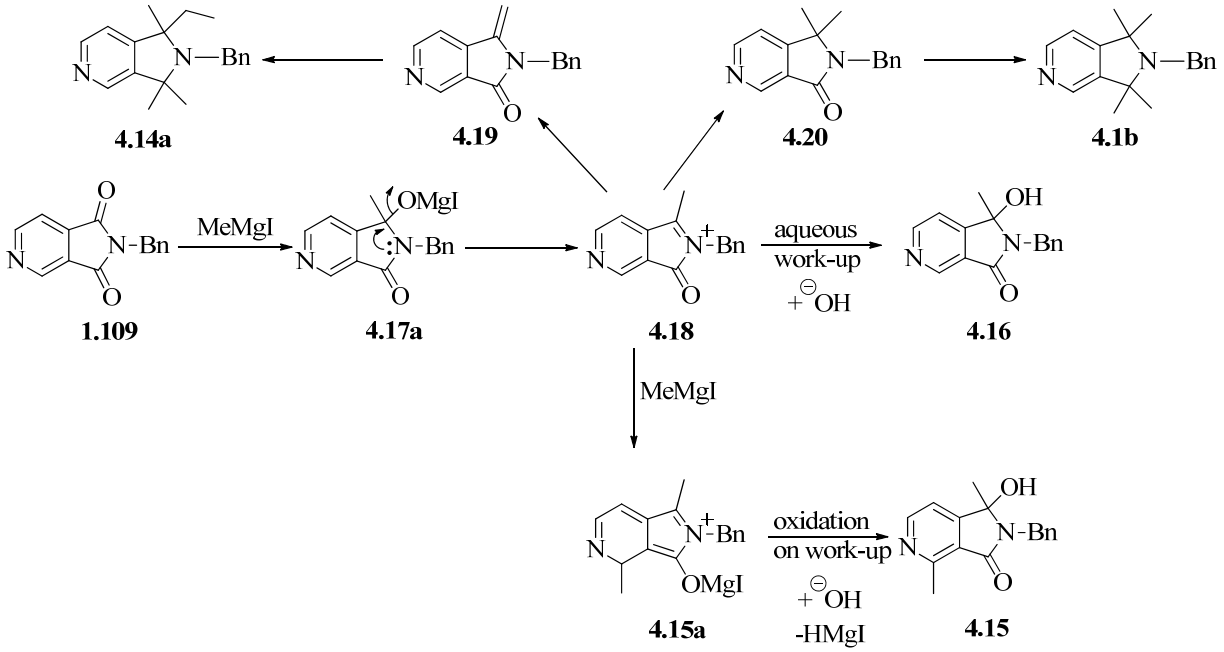
Table 4.5 HPLC product ratios obtained from the reaction of imide **1.109** with excess MeMgI.

Entry	Eqv. MeI	Eqv. Mg	Reaction Temp.[°C]	Reaction time [h]	HPLC product ratio (%)			
					4.16	**4.15**	**4.14**	**4.1b**
1	6.0	8.0	110	3	57	43	-	-
2	6.0	8.0	110	5	41	27	10	22
3	6.0	8.0	110	15	60	35	2	3
4	6.0	8.0	110	24	48	30	19	3
5	6.0	8.0	110	72	28	37	31	4
6	6.0	8.0	110	120	28	40	27	5

Hydroxy amide product **4.16** would be expected to arise from either an iminium ion **4.18** on aqueous work up or from hydrolysis of the corresponding magnesium alkoxide **4.17a** (Scheme 4.11).[140] Iminium ion **4.18** may undergo ring methylation (Scheme 4.11) to form **4.15a**, which is believed to be a non-reactive product during the reaction and later may undergo rearomatization and oxidation during the work up[58] to form **4.15**. On the other hand, any of the tetraalkyl adducts (**4.1b** and **4.14**) do not contain methylated pyridine rings and this also shows that **4.15** represents a "dead-end" side reaction.

It was hypothesised that ethyltrimethyl adduct **4.14a** (or **4.14b**) could be derived (similar to the isoindolinic analogous **1.82** in chapter 3) starting from an exocyclic alkene **4.19** in the presence of unreacted iodomethane in the reaction mixture (Scheme 4.11). Since exocyclic amide **4.19** is likely to be derived from the initial precursor intermediate **4.18** (which is also the precursor of **4.16**), and the regio-

isomerism of **4.16** was previously confirmed by HMBC (Figure 4.6) as shown in Scheme 4.11, it was concluded that ethyltrimethyl adduct would presumably represent regio-isomer **4.14a** instead of **4.14b**. Further increase of refluxing time from 5 hours to 120 hours decreased the level of **4.1b**, while the level of **4.14a** increased. Presumably at higher temperatures, an exocyclic alkene such as **4.19** could be produced efficiently due to the increased elimination of H^+ from **4.18** or –Mg(OH)I from **4.17a**. Increased conversion of **4.18** into **4.19** (and later into **4.14a**) and **4.15** may reduce the yield of **4.1b** as a consequence.



Scheme 4.11 One possible pathway of forming **4.16**, **4.15**, **4.1b** and **4.14a** during the refluxing of imide **1.109** with excess MeMgI (110 °C).

It is interesting that the Grignard ethylation of imide **1.109** did not lead to the desired tetraethyl adduct **4.1a**, but the methylation of **1.109** gave the desired target product **4.1b**. It is however possible that iminium ion **4.18** would form a 1,1-dimethyl adduct (**4.20**), analogous to the 1,1-dimethyl adduct **3.2** isolated during the tetramethylation of phthalimide **1.75** (See chapter 3). Since dimethyl amide **3.2** converted to the

tetramethyl (isondoline) adduct **1.76** (in good yield), it was hypothesised that one way of forming tetramethyl adduct **4.1b** could be *via* a 1,1-dimethyl adduct such as **4.20**. (Since **4.20** was not observed or isolated as a product in the Grignard reactions shown in Table 4.5, it was speculated that **4.20** could have been converted totally to **4.1b** during the reaction).

Further improving the yield of tetramethyl adduct **4.1b** was carried out by changing the experimental conditions (Equivalents EtMgI, reaction temperature and reaction time) and analysing the mixtures by HPLC. The relative HPLC yields of **4.1b** obtained under different experimental conditions are tabulated in Table 4.6 below. The best HPLC relative yield for **4.1b** (32%) was obtained by refluxing imide **1.109** with 8 equivalents of MeMgI in toluene for 72 hours (Entry 3, Table 4.6). Further increasing the scale of the reaction up to 15.0 mmol (Entry 4, Table 4.6) gave a relative HPLC yield of 23% and isolated yield of 18% for **4.1b**. The isolated yield of tetramethyl adduct **4.1b** was lower than the HPLC yield due to the formation of a brown-colored toluene-insoluble mass in the reaction mixture, which is not visible in the HPLC chromatogram (Figure 4.9). Since the target synthesis (tetramethylpyridine adduct **4.1b)** was achieved succesfully, further investigations were not pursued to identify the brown-colored insoluble mass.

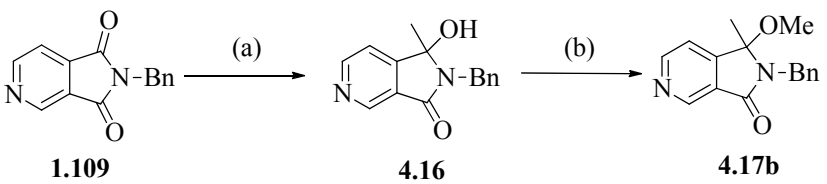
Table 4.6 Optimization of reaction conditions of entry 2 in order to improve the yield of tetramethyl adduct **4.1b**

Entry	Eqv. MeI	Eqv. Mg	Reaction Temp.[°C]	Reaction time [h]	HPLC product ratio (%)			
					4.16	**4.15**	**4.14a**	**4.1b**
1	6.0	8.0	140	5	60	30	2	8
2	6.0	8.0	110	5	34	40	14	12
3	8.0	10.0	110	72	20	22	26	32
4	8.0	10.0	110	72	32	31	14	23

Entry 3: 1.00 g scale reaction; **Entry 4:** 3.50 g scale reaction

4.3.2.2.2 Optimizing the yield of tetramethyl adduct **4.1b** by modifying the substrate **1.109**

Optimization of the yield of **4.1b** was next attempted by the method used to improve the yield of tetraethyl (isoindoline) adduct **1.94**[140] that is, from methyl hydroxyl amide **4.16** and methylmethoxy amide **4.17b**. Synthesis of **4.16** from imide **1.109** was carried out similarly to the synthesis of methylhydroxy amide **3.3** (see chapter 3). Treatment of imide **1.109** (in toluene) with 2.5 equivalents of MeMgI (prepared in Et_2O) and stirring the mixture at room temperature for 2 hours gave **4.16** with a yield of 45%, followed by quenching the Grignard reaction mixture with aqueous NH_4Cl and isolating the product by column chromatography (hexane/ethylacetate 1:1). Although this reaction was carried out similar to the previously optimized experimental conditions (which gave 94% isolated yield for the isoindoline methylhydroxy amide **3.3** and 86% isolated yield for isoindoline ethylhydroxy amide **2.2**; See Chapters 2 & 3), the yield of the pyridine annulated methylhydroxy amide **4.16** was lower presumably due to the formation of brown-coloured toluene insoluble mass (which was also observed during the synthesis of **4.1b** from Grignard methylation of **1.109**). Further attempts to improve the yield of this step were not undertaken as this brown-coloured mass could be similar to the purple-coloured oligomeric material (Chapter 3) which diminished the yield of the target **1.76**. Therefore further improvements of the yield of **4.16** were not attempted. Recrystallization of the crude product of **4.16** (hexane and ethylacetate) gave a crystal yield of 40%.



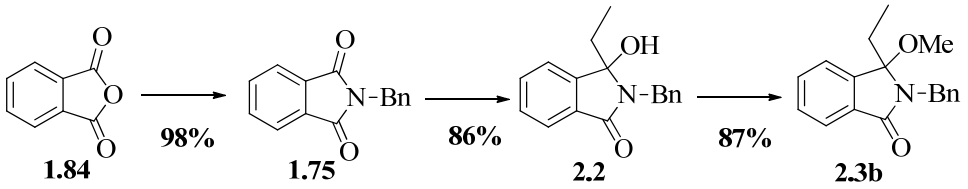
1.109 **4.16** **4.17b**

Scheme 4.12 Synthesis of **4.16** and **4.17b** starting from imide **1.109**.

Reagents and conditions: (a) **1.109**, MeMgI (2.5 equiv.), toluene, rt, 2 h, 40%. (b) **4.16**, MeI (4.0 equiv.), NaOH (4.0 equiv.), THF, rt, 24 h, 75%.

Methylation of methylhydroxy amide **4.16** with excess MeI/NaOH in THF at room temperature for 1 day gave the target methyl methoxy amide **4.17b** in a crude yield of 84% (75% recrystallised yield). When the overall synthetic yields of the modified starting materials **2.2** (ethylhydroxy amide) and **2.3b** (ethylmethoxy amide) starting from phthalimide **1.75** were compared[140] with that of the corresponding pyridine methylhydroxy amide **4.16** and methylmethoxy amide **4.17b** (starting from imide **1.109**), the latter percentages were unexpectedly lower (Scheme 4.12). Therefore Grignard tetramethylation on **4.16** and **4.17b** was not pursued as the substrate synthesis was low yielding.

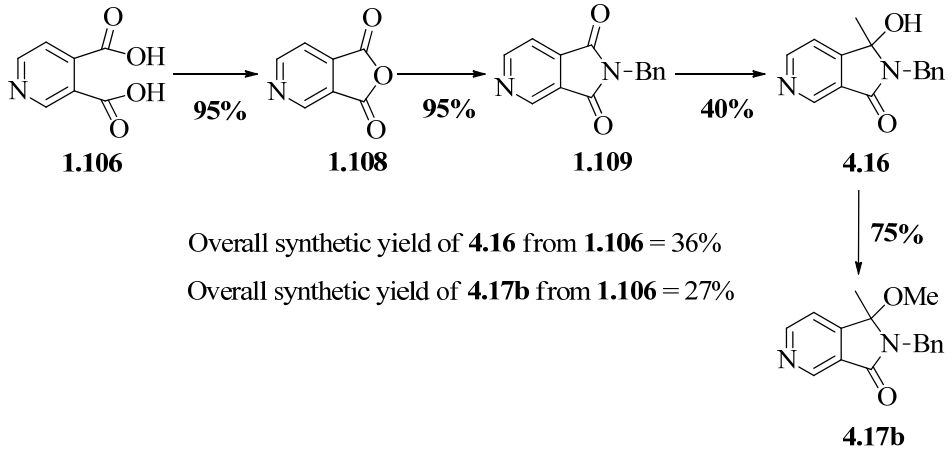
Scheme A



Overall synthetic yield of **2.2** from **1.84** = 84%[140]

Overall synthetic yield of **2.3b** from **1.84** = 73%[140]

Scheme B



Overall synthetic yield of **4.16** from **1.106** = 36%

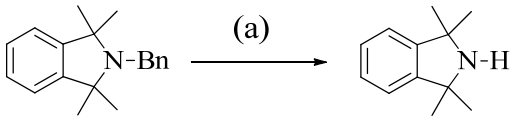
Overall synthetic yield of **4.17b** from **1.106** = 27%

Scheme 4.13 Comparison of the synthetic yields of **4.16** and **4.17b** with that of **2.2**[140] and **2.3b**[140] starting from commercially available **1.106** and **1.84**

4.3.3 Hydrogenation of tetramethyl adduct **4.1b**.

4.3.3.1 Background

Hydrogenation of **4.1b** was carried out similar to the Griffiths' literature procedure for hydrogenation of *N*-benzyl-1,1,3,3-tetramethylisoindoline **1.76** (Scheme 4.14).[35]



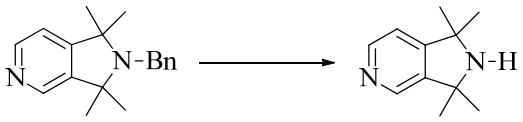
1.76 **1.77**

Scheme 4.14 Synthesis of 1,1,3,3-tetramethylisoindoline **1.77** from the hydrogenation of **1.76**[35]

Reagents and conditions: (a) **1.76** in AcOH, H_2/Pd/C at room temperature, 3 h, 60 lb/in^2, then 10% NaOH and ether, 96%.[35]

4.3.3.2 Synthesis of 1,1,3,3-tetramethyl-1,3-dihydro-1*H*-pyrrolo [3,4-*c*]pyridine 4.2

Starting material **4.1b** dissolved in MeOH was reacted with H_2 gas in the presence of catalyst based on palladium at 10 wt. % on carbon for 3-4 hours. Complete conversion of the starting substrate **4.1b** to a new, more polar compound was verified by TLC analysis.



4.1b **4.2**

Scheme 4.15 Synthesis of **4.2** from the hydrogenation of **4.1b**.

Reagents and conditions: 4.1b, H_2/Pd/C, MeOH, rt, 3-4 h, 95%.

Removal of charcoal impurities by celite filtration and evaporation of methanol under reduced pressure gave an oily material which was later purified by column chromatography (ethyl acetate:EtOH, 7:1). This was identified as title amine **4.2** by

NMR spectroscopy and Mass spectrometry, and gave an overall isolated yield of 95%. The five aromatic carbons of the pyridine ring of **4.2** were assigned with the help of HSQC and DEPT spectra. According to the HSQC spectra (Figure 4.11), the correlation between C4 and the H attached to C4 is indicated by correlation B, because the related peak in the ^{1}H-NMR spectrum is a singlet. The other two hydrogens (H^6 and H^7) appear as two doublets as they are expected to be coupled from each other. The correlation spots A and C represent the correlations between C7 and its H atom and C6 and its H atom respectively (Figure 4.11).

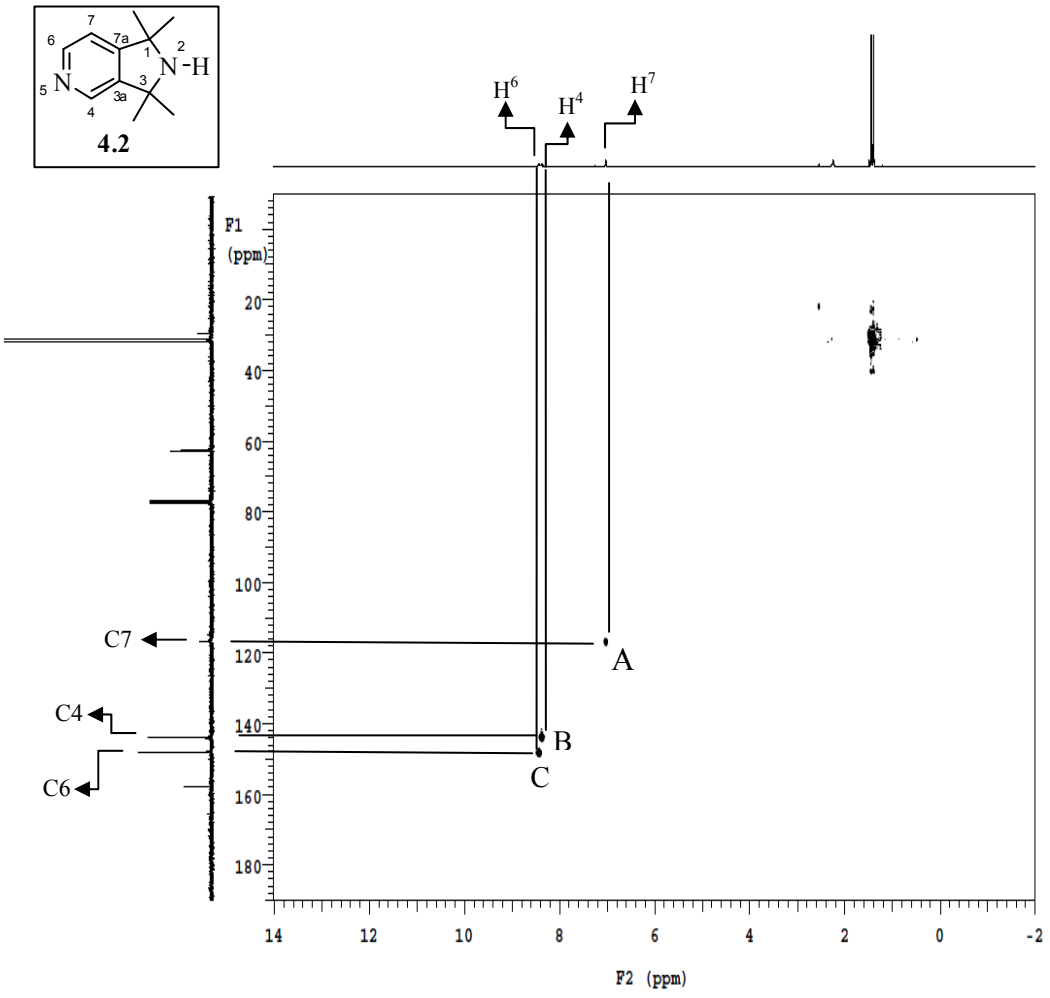
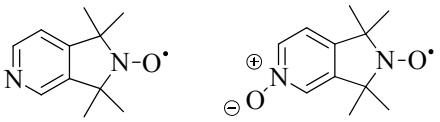


Figure 4.11 HSQC spectrum of product 4.2.

Quartenary aromatic carbons C7a and C3a were identified by means of a DEPT spectrum as one of the quartenry carbons appeared relatively smaller compared to the other.

4.3.4 Oxidation of tetramethyl amine 4.2 to synthesize 1,1,3,3-tetramethyl-1,3-dihydro-1*H*-pyrrolo [3,4-*c*]pyridine-2-yloxyl radical 4.3

Oxidation of **4.2** was carried out in a similar way to the Griffiths' patented procedure.[35] Starting substrate **4.2** dissolved in MeOH/MeCN was reacted with $Na_2WO_4.2H_2O$ and $NaHCO_3$ and aqueous H_2O_2 (30%) for 48 hours. Analysing the reaction mixture by TLC (ethyl acetate:EtOH, 7:1) indicated a new compound (which was relatively polar) along with some unreacted starting material (**4.2**). The reaction mixture was diluted with deionized water and extracted with CH_2Cl_2 to give yellow crude. Isolation of the relatively polar compound by column chromatography (ethyl acetate:EtOH, 7:1) and analysis of it by mass spectrometry and EPR (characteristic three line spectrum) showed the formation of heterocyclic nitroxide **4.3** with an isolated yield of 80%.



4.3 **4.3a**

Figure 4.12 Different nitroxides formed from the oxidation of **4.2**.

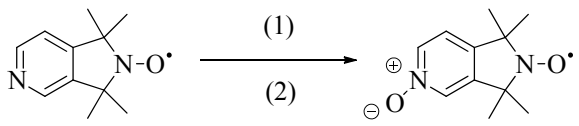
A relatively large scale reaction (0.100 g scale) of this was carried out with an extended stirring time (120 hours) in the expectation that the unreacted starting material would convert to the target nitroxide. Analysis of the reaction mixture by TLC showed that the spot which was speculated to be unreacted starting material had become stronger (under UV) while the spot of nitroxide **4.3** had become weaker. Isolation of the stronger spot by column chromatography (ethyl acetate:EtOH, 7:1) and analysis by EPR indicated a characteristic three line spectrum similar to the EPR spectrum of **4.3**. From this, it was concluded that another nitroxide (similer to **4.2** by retention on TLC) had been formed in the reaction mixture. Mass spectrometry and

elemental analysis identified the unknown nitroxide as **4.3a**, which was the major product (85% isolated yield) of Entry 2 (Table 4.7). Treatment of nitroxide **4.3** with excess aqueous H_2O_2 (30%) and $Na_2WO_4.2H_2O$ catalyst gave nitroxide **4.3a** after stirring 48 hours (Scheme 4.17). However, this conversion was achieved within 2 hours, when **4.3** in DCM was treated with *m*-CPBA at room temperature (Scheme 4.16).

Table 4.7 Controlling the isolated % yields of the nitroxides **4.3** and **4.3a** by changing the reaction time.

Entry	Equiv. H_2O_2	Equiv. catalyst	Reaction Temp[°C]	Reaction time [h]	Isolated % yields **4.3**	**4.3a**
1	3.3	0.03	rt	48	80	17
2	3.3	0.03	rt	120	6	85

Since the H_2O_2 oxidation of **4.2** was a slow conversion, oxidation of amine **4.2** using *m*CPBA (*m*-chloroperbenzoic acid) was attempted according to the method reported by Fairfull-Smith and co-workers.[109] Amine **4.2** was treated with *m*CPBA in DCM at 0 °C and the temperature of the reaction mixture was allowed to reach room temperature. Stirring the reaction mixture at room temperature for 3-4 hours gave **4.3a** as the only nitroxide present in an isolated yield of 81%.

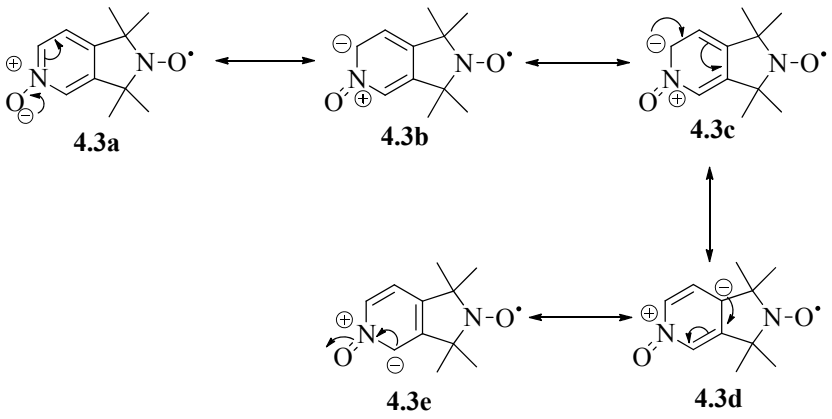


4.3 **4.3a**

Scheme 4.16 Conversion of **4.3** to **4.3a**

Reagents and conditions: (1) **4.3**, MeOH/MeCN, $H_2O_2/Na_2WO_4.2H_2O/NaHCO_3$, rt, 48 h; or (2) **4.3**, DCM, m-CPBA, rt, 2 h.

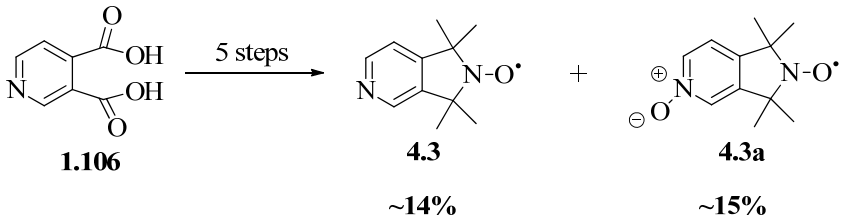
Pyridine systems can be readily oxidized to pyridine *N*-oxides with reagents such as *m*-CPBA and H_2O_2.[131] These pyridine *N*-oxides are stable dipolar species wherein the electrons on oxygen are delocalized around the pyridine ring, raising the HOMO of the molecule. This feature has made pyridine *N*-oxides a reactive synthetic tool for electrophilic substitution reactions such as nitration.[131] The resonance stabilization of **4.3a** is shown below (Scheme 4.17).



Scheme 4.17 Resonance stabilization of the pyridine *N*-oxide **4.3a**.

4.3.5 Final overview of the overall yields of the nitroxides **4.3** and **4.3a**

The overall synthetic yields of the two nitroxides, **4.3** and **4.3a** starting from pyridine-3,4-dicarboxylic acid **1.106** are shown below (Scheme 4.18). This novel approach for synthesizing pyridine-annulated heterocyclic nitroxides (**4.3** and **4.3a**) is short and convienient compared to the previous electrocyclic approaches (see Chapter 1) and the overall synthetic yields are much improved.



Scheme 4.18 Overall synthetic yields of the heterocyclic nitroxides **4.3** and **4.3a** starting from **1.106**.

These two nitroxides were preliminarily identified by EPR spectroscopy (Figure 4.13) which gave characteristic three line spectra. (see Chapter 6 for other characteristic data).

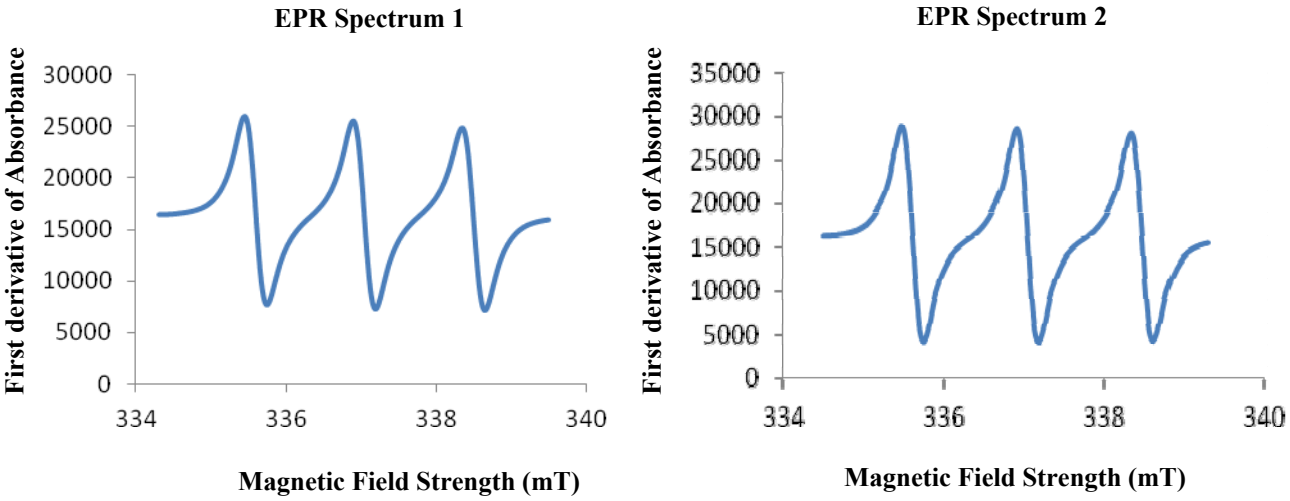


Figure 4.13 EPR spectra of the nitroxides **4.3** (Spectrum 1) and **4.3a** (Spectrum 2).

4.4 CONCLUSIONS

The main aim of this part of the project was to introduce the synthesis of tetraalkylated pyridine-annulated heterocyclic nitroxides *via* a short and convenient pathway. It is clear that Grignard reaction using EtMgI at higher temperature (starting from imide **1.109**) gives rise to a number of structurally modified derivatives of imide **1.109**, but not the desired tetraethyl adduct **4.1a**. None of these derivatives appears to be intermediates on the pathway to the desired tetraethyl adduct **4.1a**. Attempts to synthesize **4.1a** by modifying the experimental conditions and the structure of the starting material **1.109** were not successful, due to the generation of Grignard products not further reacting under the conditions employed. Interestingly, methylation of imide **1.109** with excess MeMgI in toluene at reflux gave the desired tetramethyl adduct **4.1b** along with three side products. Although the yield of **4.1b** was limited by two side-products and an insoluble brown coloured

material, the final synthetic yield of the tetramethyl adduct **4.1b** was improved up to 18% (by modifying experimental conditions followed by the HPLC analysis). Subsequent hydrogenation of tetramethyl adduct **4.1b** with H_2/Pd/C to produce amine **4.2** (95% yield) and oxidation of **4.2** with aqueous H_2O_2 (30%)/$Na_2WO_4.2H_2O$/$NaHCO_3$ gave target tetramethylpyridine nitroxides **4.3** in a yield of 80% within 48 hours. Extending the reaction time (to 120 hrs.) of the oxidation reaction further converted the target nitroxide **4.3** into the pyridine-*N*-oxide version **4.3a** in a yield of 85%.

This approach improved the overall synthetic yields of novel pyridine-annulated tetramethyl nitroxides (**4.3** and **4.3a**) up to ~15% via a short (5 steps) and convenient pathway, in comparison to the existing synthetic approaches based on electrocyclic reactions.

4.5 MANUSCRIPT OF THE WORK RELATED TO CHAPTER 4

Manuscript of the work related to this Chapter is in preparation...!

Chapter 5: Conclusions

5.1 CONCLUSIONS

The two main goals of this PhD were i) to examine and improve the exhaustive Grignard alkylation of *N*-benzylphthalimide **1.75** and ii) to exploit the knowledge gained through this investigation in order to develop a short and convenient pathway for the synthesis of heteroaromatic nitroxides.

In Chapter 2 two aspects were mainly discussed, the factors diminishing the yield of tetraethyl adduct **1.94**, and some novel approaches to improve the yield. The initial aim was the structurally modification of the phthalimide **1.75** with a good leaving group such as a triflate, mesylate or acetylate by using ethylhydroxyamide **2.2** as the starting material, however, this was unsuccessful due to the formation of exocyclic alkene **2.6**. The modification of phthalimide **1.75** with a less effective leaving group (such as a methoxy group) proved successful by synthesising ethylmethoxy amide **2.3b** in a high yield. Reacting methoxyamide **2.3b** with excess EtMgI in refluxing toluene for an extended duration gave an isolated yield of 55% (the best isolated yield) for tetraethyl adduct **1.94**. When the overall yield of tetraethyl adduct **1.94** was calculated over three steps starting from phthalimide **1.75**, it gave 41% which is similar to the yield of tetraethyl adduct (**1.94**) over one step in the existing Griffiths approach. Formation of high levels of hitherto-unreported side products such as diethyl amide **2.11** and ethylhydroxy amide **2.2** during this reaction suggests the influence of an improved leaving group, but has limited the yield of tetraethyl adduct **1.94** due to the unavoidable conversion of ethylhydroxy amide **2.2** into **2.11** and **2.12**, which both are non-reactive intermediates under the Grignard conditions.

As ethylhydroxy amide **2.2** represents the key initial intermediate to the desired tetraethyl adduct **1.94**, product **2.2** was treated with excess EtMgI in refluxing toluene for an extended time. This reaction gave a much improved isolated yield (57%) for tetraethyl adduct **1.94** with an overall yield of 49% over two steps starting from phthalimide **1.75**. This reaction also reduced formation of diethyl amide **2.11** and monoethyl reduction product **2.12** was not observed. This implies that the formation of tetraethyl adduct **1.94** from ethylhydroxyamide **2.2** may occur *via* a mechanism which is unlikely to go through iminium ion **2.5**. Therefore it was hypothesised that ethylhydroxyamide **2.2** (as a substrate) may go through literature-reported O-Mg-O bridging complex **2.16** in order to efficiently produce an intermediate such as iminium ion **2.13b**. Formation of diethylamide from the Grignard ethylation of **2.2** prompted us to examine if side-products such as diethylamide **2.11** and monoethylamide **2.12** were present in standard Grignard ethylation of *N*-benzylphthalimide **1.75**. Formation of considerable amount of **2.11** and small amount of **2.12** in the Grignard ethylation of phthalimide **1.75** indicate the likely cause of the limited yield of tetraethyl adduct (**1.94**). Starting from a partially-ethylated starting material such as ethylhydroxy amide **2.2** provided practically higher yields even though two steps are required.

A similar analysis was carried out to investigate the analogous Grignard methylation of *N*-benzylphthalimide **1.75** and improve the yield of **1.76**, however, this examination gave complex results compared to the ethylation of phthalimide **1.75**. Upon a change of the work-up procedure to incorporate aqueous NH_4Cl, three previously unreported side-products, exocyclic alkene **3.1**, dimethyl amide **3.2** and methylhydroxy amide **3.3**, were isolated in considerable yields along with (previously reported) tetramethyl adduct **1.76** and ethyltrimethyl adduct **1.82**. Further

investigations were undertaken to identify the pathways leading to these side-products to determine if they could be influencing the overall yield of tetramethyl adduct **1.76**.

When Grignard methylation reaction was undertaken on exocyclic alkene **3.1**, the reaction did not produce desired tetramethyl adduct **1.76**, but gave rise to the unsubstituted mix alkylated adduct **1.82**, implying that exocyclic alkene **3.1** may be the precursor intermediate of **1.82**. Grignard methylation on side-prodcuts such as dimethyl amide **3.2** and methylhydroxy amide **3.3** showed that both **3.2** and **3.3** are intermediates on the pathway to the desired tetramethyl adduct **1.76**. Undertaking Grignard methylation reactions on side products **3.1** and **3.3** also generated an incompletely characterisable purple material which was characteristic of the Grignard methylation of phthalimide (**1.75**). This implies that exocyclic alkene **3.1** is a precursor (if not the direct precursor) to the formation of aforementioned purple-coloured oligomeric material. Since Grignard methylation on **3.3** also produced the purple-colored oligomeric material, it was concluded that this purple product generation might have occurred through exocyclic alkene **3.1** as the dehydration of methylhydroxy amide **3.3** could form alkene **3.1**.

Improving the yield of the tetramethyl adduct **1.76** through modifications of the structure of phthalimide **1.75** (such as utilizing methylhydroxy amide **3.3** or methylmethoxy amide **3.4b** as starting material) or the reaction conditions were also inherently difficult due to the increased formation of iminium ion **3.5** which led to the increased amounts of dead-end side products (**1.82** and **3.1**) and the purple oligomer. Attempts to elucidate the structure of the purple oligomeric material were largely unsuccessful due to its complexity and limited solubility; however, a few structural features were revealed which gave some indications as to the nature of the

product (See Chapter 3 for detailed discussion). The experiments carried out in Chapter 3 showed clearly that the generation of 'dead-end side products' (ethyltrimethyl adduct **1.82** and exocyclic alkene **3.1**) and a purple oligomeric mixture limit the overall yield of tetramethyl adduct **1.76** starting from phthalimide **1.75**. Finally, the knowledge gained in this examination was useful in the development of pyridine-annulated heterocyclic nitroxides in chapter 4.

Synthesis of starting imide **1.109** (for the synthesis of pyridine-annulated heterocyclic nitroxide **4.3**) starting from commercially available 3,4-pyridinedicarboxylic acid **1.106** was achieved with an overall yield of ~90% over two steps. Grignard ethylation of imide **1.109** with six equivalents of EtMgI in refluxing toluene for five hours gave five products; three diethyl amide derivatives (**4.4**, **4.5**, **4.6**) and two ethylhydroxy amide derivatives (**4.7** and **4.8**), but the desired tetraethyl adduct **4.1a** was not observed in the reaction mixture. Considerable efforts were undertaken to convert imide **1.109** into **4.1a** by varying the experimental conditions (reaction temperature, reaction time and equivalence of EtMgI) and modifying the imide **1.109** into ethylhydroxy amide **4.8**, but none of these reactions gave the desired tetraethyl adduct **4.1a**. Based on previous literature reports of similar reactions,[140,58] it was concluded that diethyl amide derivatives or ethylhydroxy amide derivatives formed in the mixture do not appear to be intermediates on the pathway to the desired tetraethyl adduct **4.1a**.

Treatment of starting imide **1.109** with excess MeMgI in toluene at reflux for five hours surprisingly gave tetramethyl adduct **4.1b** in a relatively low yield along with three other side products; ethyltrimethyl adduct **4.14**, methylhydroxy amide **4.16** and its ring methylated derivative **4.15**. The yield of the tetramethyl adduct **4.1b** was improved to 18% by increasing the equivalents of MeMgI in refluxing toluene for

extended times. However, further attempts to improve the yield of the desired tetramethyl adduct **4.1b** by modifying imide **1.109** structurally (such as methylhydroxy amide **4.16** and methylmethoxy amide **4.17b**) were largely unsuccessful as synthesising these structures (**4.16** and **4.17b**) were low yielding. Conversion of tetramethyl adduct **4.1b** to the corresponding amine **4.2** was achieved by hydrogenation (H_2/Pd/C in MeOH) in a yield of 95%. Amine **4.2** was oxidized to the target heterocyclic nitroxide **4.3** in a yield of 80% by reacting with H_2O_2 /$Na_2WO_4.2H_2O$/$NaHCO_3$ in MeOH (48 h). When the same reaction was extended to five days, nitroxide **4.3** was further oxidized to another novel nitroxide **4.3a** (pyridine-*N*-oxide) in a yield of 85%. The final overall synthetic yield of pyridine annulated heterocyclic nitroxides (**4.3** and **4.3a**) was achieved in ~14-15%. This achievement was comparatively higher in yield-wise compared to the previously synthesized pyridine-annulated nitroxides (5-10%).[89,93,123,101,102] The reported synthesis (see Chapter 4) of novel pyridine-annulated nitroxides *via* a novel five-step route gives practically improved yields on preparative scale over the existing synthesis of substituted heterocyclic nitroxides.

5.2 FUTURE WORK

All the goals of this PhD work were achieved successfully; however, the knowledge gained during this project can be exploited in some future explorations. Improving the yields of the alkylation of imides using partially-alkylated derivatives could be a useful method to improve the yields of some existing low-yielding methodologies. One synthetic route where this step-wise addition synthesis could be applied to improve the synthetic yield of tetramethyl adduct is the methylation of *N*-benzyl-1,8-naphthalimide **4.11**. Blinco and co-workers have improved the yield of the tetramethyl adduct **5.2** in 15% in a single step,[152] however it is worth attempting to

use methylhydroxy amide derivative **5.1a** or the methylmethoxy amide derivative **5.1b** as starting materials to improve the yield of the tetramethyl adduct **5.2**.

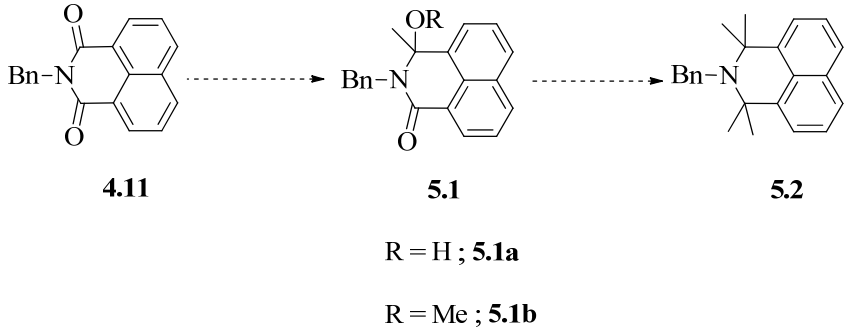


Figure 5.1 Proposed synthetic pathway to obtain improved yields for tetramethyl adduct **5.2**.

The novel five-step approach used to synthesize pyridine-annulated heterocyclic nitroxides could be exploited in synthesizing furan-annulated heterocyclic nitroxides such as **5.4** in improved yields. The furan-annulated pyrroline nitroxide **5.4** has been synthesized previously by Hideg and co-workers.[123] They have carried out the synthesis of **5.4** starting from commercially available esterified nitroxide **87** *via* nine steps with a very poor yield (~0.6%).[89,123] One possibility to improve the yield of this furan-annulated heterocyclic nitroxide **5.4** could be initiation of the synthesis from commercially available furan-3,4-dicarboxylic acid **5.3**, *via* a Grignard approach (Figure 5.2).

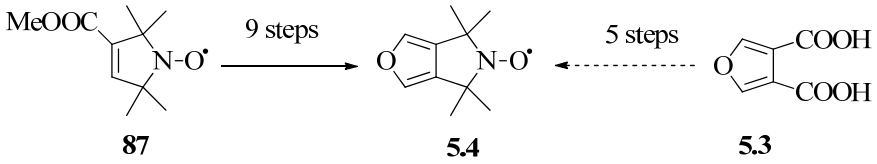
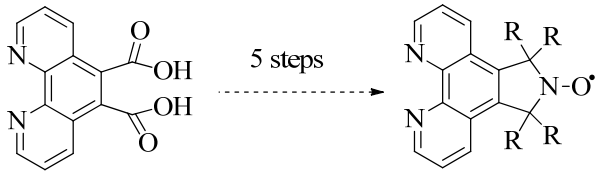


Figure 5.2 Exsiting synthetic methodology (9 step route)[89,123] and the proposed synthetic methodology (5 step route) for the synthsis of furan annulated nitroxide **5.4**.

There is also the potential to synthesise another novel heterocyclic nitroxide which contains two pyridine rings; that is phenanthroline analogues. This synthesis could be carried out similarly to the synthesis of pyridine-annulated nitroxides (such as **4.3**) starting from commercially available 1,10-phenanthroline-5,6-dicarboxylic acid **5.5** *via* a Grignard approach. The proposed synthetic pathway is shown below (Figure 5.3).



5.5 **5.6**

Figure 5.3 Proposed synthetic pathway for phenanthroline based nitroxides **5.6**.

Pyridine is extremely rich in chemistry, so it is not surprising that it has many applications.[131] Since the target nitroxides, **4.3** and **4.3a** are pyridine annulated heterocyclic systems, it would be of interest to apply some previously reported chemistry of pyridine containing compounds to these nitroxides as well. A well-known method of activating pyridine systems for both electrophilic and nucleophilic substitution reactions is converting the N of the pyridine ring into *N*-oxide.[131] As one of the nitroxides generated in this project was a pyridine-*N*-oxide (**4.3a**), this would be an interesting synthetic tool for a range of novel heterocyclic nitroxide derivatives. Pyridine *N*-oxides can also be converted to the parent pyridine systems again.[131]

Pyridine is also a good monodentate ligand with good σ-donor capabilities.[121] Pyridine can be coordinated to metals such as Sn^{4+}, Zn^{2+}, Ni^{2+} etc. to form metal complexes. Since one of the heterocyclic nitroxides (**4.3**) has a free N atom (in the pyridine ring) which is not bound to any of the atoms, it would be an interesting ligand for some important metal complexes. All these aforementioned applications of

pyridine ring systems could be seen in a proposed pyridine-annulated target nitroxide such as **5.6** (Figure 5.3) as well.

In addition, the degradation studies, redox potentials, antioxidant properties, some biological studies etc. of these novel nitroxides (**4.3** and **4.3a**) are also important to investigate. Such studies would provide an essential endorsement for further development of the synthesis of pyridine-annulated heterocyclic nitroxides *via* the new route described here. As the experimental science does not end, it always breeds a bounty of progeny.

Chapter 6: Experimental

6.1 GENERAL METHODS

All reported synthetic work was performed at Queensland University of Technology, Brisbane, Australia.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer or Varian INOVA 400 NMR spectrometer at 400 and 100 MHz respectively. Samples for the above spectroscopic analysis were prepared in deuterated $CHCl_3$ (unless otherwise specified) and all the peaks were referenced to the solvent ($CDCl_3$; δ_H =7.26 ppm, δ_C =77.0 ppm). *J* values are given in Hz while chemical shifts (δ) are given in ppm. Specific isomers of some compounds were interpreted with the help of 2D spectra such as HMBC and NOESY.

ESI-high resolution mass spectra were obtained using an Agilent QTOF LC high resolution mass spectrometer which utilized electrospray ionisation (recorded in the positive mode). An Agilent 1100 Series diode array was connected in-line with the mass selective detector (using an m/z range of 28-1000). LC flow (100% methanol at 1 mL min-1) was introduced into the ESI interface after detection by the photo-diode-array. EI-high resolution mass spectra were recorded with an Agilent double focusing magnetic sector mass spectrometer in the positive mode.

Matrix-assisted laser desorption/ionization mass spectrum was measured at University of Queensland, Brisbane. Analysis was performed with an Applied Biosystems Voyager-DE STR Biospectrometry workstation. The instrument was operated in positive polarity in linear mode for analysis. For ionisation, 337 nm laser beams were used and the data obtained was processed with the instrument

manufacturer's Data Explorer software. MALDI-ToF-mass spectra of the purple material and the experimental details of the measurements were kindly provided by Mr. Thomas Bennett (PhD candidate, University of Queensland).

Fourier transform infrared (FTIR) spectra were collected using Nicolet 870 Nexus Fourier Transform Infrared system equipped with an attenuated total internal reflectance (ATR) accessory and Deuterated Tri-Glycine Sulfate Thermoelectric cooler detector with a single bounce diamond cell.

Melting points were determined using a Gallenkamp melting point apparatus by capillary method with a thermometer calibrated against nine compounds of known melting points.

UV/visible spectrum was recorded on a SHIMADZU-UV-1800 instrument (in DCM) by Mr. Liam Walsh (PhD candidate, Queensland University of Technology).

Elemental analysis was performed by the Microanalytical services, Department of Chemistry, University of Queensland.

Analytical HPLC was carried out on a HP Agilent 1100 HPLC instrument using a Prep-C18 scalar column (4.6 × 150 mm, 10 μm) with a flow rate of 1 mL/min in the stated mixtures of methanol and water with detection at 254 nm.

Analytical (reversed phase) MPLC was carried out on a MPLC system using Agilent Prep-C_{18} scalar column (SF10-10g, AX1409-1) with a flow rate of 1 mL/min in the stated mixtures of THF and water with detection at 254 nm.

GPC system was performed using a Waters system equipped with a Waters 1515 isocratic HPLC pump, 200 μL injection loop, and three consecutive phenomenex, phenogel 5μ columns (300x7.8 mm) (10^4 °A, 10^3 °A, 50 °A) (Effective molecular weight range of the column series: 100-3000, 1000-75 000, 5000-500 000 $gmol^{-1}$), preceded by a Phenomenex 5μ linear mixed bed guard column (50 X 7.8 mm)

(separation range: 10-10,000,000) operating at 30 °C with THF as the eluent (flow rate 1 ml/min). The instrument was fitted with a Waters 2414 refractive index (RI) detector. The size exclusion chromatogram was kindly measured by Mr. Paul Ledarhorse (PhD candidate, Queenaland University of Technology) and it was kindly interpreted by Dr. John Colwell (Research fellow, Queenaland University of Technology).

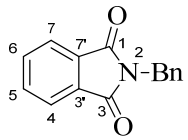
Thin-Layer Chromatography (TLC) was performed using Silica Gel 60 F_{254} TLC plates and preparative column chromatography was performed using Silica Gel 60 (230-400 mesh), unless otherwise specified.

All the solvents used were of analytical reagent (AR) grade where possible. Solvents for Grignard reactions such as toluene, xylenes and diethyl ether were dried and stored over sodium wire. Tetrahydrofuran (THF) was freshly distilled from sodium benzophenone ketal and dichloromethane (DCM) was freshly distilled from calcium hydride. Triethylamine used in experiments was dried and stored over potassium hydroxide. Solvents for chromatography were distilled prior to use. Water used in the experiments was deionised. Methanol and water used in HPLC analysis were of HPLC grade.

All the reagents were purchased from commercial suppliers and used without further purification. Starting materials such as pyridine-3,4-dicarboxylic acid was purchased from Sigma-Aldrich. Magnesium turnings utilized in the Grignard reactions were pre-dried prior to the reaction. All air sensitive reactions were carried out under argon atmosphere.

6.2 SPECIFIC EXPERIMENTAL METHODS

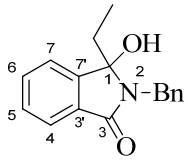
N-Benzylphthalimide (1.75)



1.75

A mixture of phthalic anhydride **1.84** (53.0 g, 0.360 mol), acetic acid (250 mL) and benzylamine (60.0 mL, 0.550 mol, 1.5 equiv.) was refluxed at 120 °C for one hour. The hot reaction mixture was then poured in to ice/H_2O (750 mL) and stirred. The resulting precipitate was filtered and recrystallised from 96% ethanol to give the title compound (white needles, 82.0 g, 96%). Mp 115-117ºC (lit.[106] mp 116 ºC); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.84 (s, 2H, benzyl-CH_2), 7.26-7.33 (m, 3H, benzyl-Ar-H), 7.43 (d, J = 7.6 Hz, 2H, benzyl-Ar-H), 7.70 (dd, J = 3.2 and 5.6 Hz, 2H, Ar-H), 7.84 (dd, J = 3.2 and 5.6 Hz, 2H, Ar-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 41.6 (benzyl-CH_2), 123.3 (benzyl-Ar-C), 127.8 (benzyl-Ar-C), 128.6 (benzyl-Ar-C), 128.7 (C_4 & C_7), 132.1 ($C_{3'}$ & $C_{7'}$), 134.0 (C_5 & C_6), 136.3 (benzyl-Ar-C), 168.0 (C=O)**;** HRMS: calcd for $C_{15}H_{11}NO_2Na$ $[M+Na]^+$ 260.0700, found 260.0430. The obtained spectroscopic data were in agreement with the previously reported data.[106]

2-Benzyl-3-ethyl-3-hydroxyisoindolin-1-one (2.2)

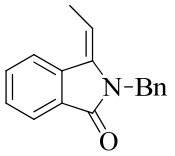


2.2

Ethyl iodide (1.00 mL, 10.0 mmol, 2.5 equiv.) was added dropwise to a suspension of pre-dried magnesium turnings (0.250 g, 10.0 mmol, 2.5 equiv.) in anhydrous

diethyl ether (15 mL). The mixture was stirred at room temperature for one hour until all the activity had subsided and was then concentrated by distillation until a temperature of 80-90ºC was reached. The reaction mixture was allowed to cool to room temperature and a solution of *N*-benzylphthalimide **1.75** (1.01 g, 4.0 mmol) in dry toluene (20 mL) was added. Once addition was completed, the mixture was stirred for 2 hours at room temperature. Saturated ammonium chloride solution (50 mL) was added and the reaction mixture was stirred until all the solids dissolved. The organic layer was separated and the remaining aqueous layer extracted with chloroform (4×50 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated at reduced pressure to give cream colored solid. Recrystallization from hexane/ethyl acetate gave the title compound **2.2** as white colored crystals (0.970 g, 86%). Mp 148-150 ºC (lit.[129] 159 ºC); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.27 (t, J = 7.2 Hz, 3H, CH_3), 2.15-2.00 (m, 2H, benzyl-CH_2), 3.37 (bs, 1H), 4.46 (d, J = 15.2 Hz ,1H, benzyl-CHH), 4.57 (d, J = 15.2 Hz, 1H, benzyl-CHH), 7.30-7.22 (m, 3H, Ar-H), 7.51-7.44 (m, 4H, Ar-H), 7.58 (t, J = 7.6 Hz, 1H, Ar-H), 7.73 (d, J = 7.6 Hz, 1H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 7.5 (CH_3), 29.3 (CH_2), 41.9 (benzyl-CH_2), 92.5 (C_1), 121.8 (Ar-C), 123.3 (Ar-C), 127.3 (Ar-C), 128.4 (Ar-C), 128.7 (Ar-C), 129.4 (Ar-C), 131.1 (Ar-C), 132.4 (Ar-C), 138.2 (Ar-C), 146.6 (Ar-C), 168.0 (C=O); HRMS: calcd for $C_{17}H_{18}NO_2$ $[MH]^+$ 268.1332; found 268.1337; Anal. Calcd for $C_{17}H_{17}NO_2$: C 76.38, H 6.41, N 5.24. Found C 76.66, H 6.43, N 5.22.

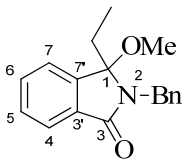
***(E)*-2-Benzyl-3-ethylideneisoindolin-1-one (2.6)**



2.6

A solution of 2-benzyl-3-ethyl-3-hydroxyisoindolin-1-one **(2.2)** (0.290 g, 1.00 mmol) and triethylamine (0.30 mL, 2.00 mmol, 2.0 equiv.) in dry dichloromethane (10 mL) was prepared and purged with argon at room temperature. Mesyl chloride (0.17 mL, 2.00 mmol, 2.0 equiv.) was added dropwise to this solution at 0 ºC over a period of 5-10 minutes. The ice bath was removed and the mixture was allowed to reach room temperature and then stirred for 1 hour. The resulting solution was washed with 2 M HCl (2×40 mL), saturated $NaHCO_3$ solution (2×40 mL) and saturated brine solution (2×40 mL). The combined layers were dried over anhydrous Na_2SO_4 and evaporated at reduced pressure to give a cream coloured solid which was purified by recrystallization (ethanol) to give **2.6** as white colored needles (0.220 g, 89%). Mp 128-129 ºC (lit.[129] 129 ºC); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.15 (d, J = 7.6 Hz, 3H, CH_3), 5.03 (s, 2H, benzyl-CH_2), 5.50 (q, J = 7.6 Hz, 1H, exocyclic-H), 7.25-7.34 (m, 5H, Ar-H), 7.53 (t, J = 7.6 Hz, 1H, Ar-H), 7.62 (t, J = 7.6 Hz, 1H, Ar-H), 7.86 (d, J = 7.6 Hz, 1H, Ar-H), 7.98 (d, J = 7.6 Hz, 1H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.1 (CH_3), 42.9 (benzyl-**C**H_2), 107.2 (CH_3-**C**H=), 123.5 (Ar-C), 123.6 (Ar-C), 126.9 (Ar-C), 127.2 (Ar-C), 128.56 (Ar-C), 128.64 (Ar-C), 130.3 (Ar-C), 131.9 (Ar-C), 135.5 (Ar-C), 135.7 (Ar-C), 137.2 (Ar-C), 166.5 (C=O); HRMS: calcd for $C_{17}H_{16}NO$ $[MH]^+$ 250.1226, found 250.1236; Anal. Calcd for $C_{17}H_{15}NO$: C 81.90, H 6.06, N 5.62. Found C 81.78, H 6.11, N 5.60. The stereochemistry around the double bond was confirmed using a NOESY spectrum.

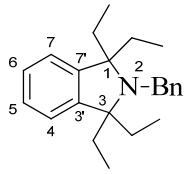
2-Benzyl-3-ethyl-3-methoxyisoindolin-1-one (2.3b)



2.3b

Finely ground sodium hydroxide (0.602 g, 15.0 mmol, 4.0 equiv.) was added to a solution of 2-benzyl-3-ethyl-3-hydroxyisoindolin-1-one **(2.2)** (0.950 g, 4.0 mmol) in dry THF (15 mL) under an atmosphere of argon. Iodomethane (1.00 mL, 16.0 mmol, 4.0 equiv.) was added dropwise and the mixture was stirred for 1.5 days at room temperature. After evaporating to dryness, the obtained residue was dissolved in CH_2Cl_2 (25 mL) and washed with deionized water (2×25 mL). The CH_2Cl_2 layer was dried over anhydrous Na_2SO_4 and concentrated at reduced pressure. The resulting residue was purified by recrystallization (hexane) to give **2.3b** as white crystals (0.870 g, 87%). Mp 62- 64 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.30 (t, J = 7.6 Hz, 3H, CH_3), 2.04-2.14 (m, 2H, CH_2), 2.58 (s, 3H, CH_3), 4.52 (d, J = 14.8 Hz, 1H, benzyl-CHH), 4.67 (d, J = 14.8 Hz, 1H, benzyl-CHH), 7.25-7.34 (m, 3H, Ar-H), 7.40 (td, J = 7.4, 0.88 Hz, 1H, Ar-H), 7.50-7.61 (m, 3H, Ar-H), 7.89 (td, J = 7.5, 0.88 Hz, 1H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 7.4 (CH_3), 29.4 (CH_2), 42.1 (benzyl-CH_2), 50.3 (O-CH_3), 96.9 (C_1), 122.0 (Ar-C), 123.5 (Ar-C), 127.4 (Ar-C), 128.3 (Ar-C), 129.2 (Ar-C), 129.6 (Ar-C), 132.2 (Ar-C), 133.0 (Ar-C), 137.9 (Ar-C), 143.0 (Ar-C), 168.4 (C=O); HRMS: calcd for $C_{18}H_{20}NO_2$ $[MH]^+$ 282.1489, found 282.1498; Anal. Calcd for $C_{18}H_{19}NO_2$: C 76.84, H 6.81, N 4.98. Found C 76.70, H 6.82, N 4.91.

2-Benzyl-1,1,3,3-tetraethylisoindoline (1.94) from 2-benzyl-3-ethyl-3-methoxyisoindolin-1-one (2.3b)



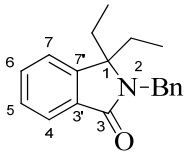
1.94

Ethyl iodide (0.20 mL, 2.00 mmol, 6.0 equiv.) was added dropwise to a suspension of pre-dried magnesium turnings (0.100 g, 3.00 mmol, 8.0 equiv.) in anhydrous diethyl ether (12 mL). The mixture was stirred at room temperature for one hour and then concentrated by distillation until a temperature of 80-90 ºC was reached. The reaction mixture was allowed to cool to 64 ºC and a solution of 2-benzyl-3-ethyl-3-methoxyisoindolin-1-one **(2.3b)** (0.100 g, 0.400 mmol) in dry toluene (10 mL) was added. Once the addition was completed, the mixture was refluxed at 110 ºC for 5 days. Saturated ammonium chloride solution (50 mL) was then added and the mixture was stirred until all the solids had dissolved. The toluene layer was separated and evaporated to dryness. The remaining aqueous layer was extracted with chloroform (4×50 mL). The combined chloroform layers were dried over anhydrous Na_2SO_4 and concentrated at reduced pressure. The resulting residues from the toluene and ethyl acetate layers were combined and purified by column chromatography (hexane:ethyl acetate, 4:1) to give **1.94** as a white solid (0.060 g, 55%). Mp 72-74 ºC (lit.[147] mp 76 ºC); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.79 (t, J = 7.6 Hz, 12H, 4 x CH_3), 1.53-1.59 (m, 4H, 2 x CH_2), 1.92-1.97 (m, 4H, 2 x CH_2), 4.03 (s, 2H, benzyl-CHH), 7.06-7.09 (m, 2H, Ar-H), 7.21-7.23 (m, 2H, Ar-H), 7.26-7.34 (m, 3H, Ar-H), 7.47 (d, J = 6.0 Hz, 2H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.8 (CH_3), 30.5 (CH_2), 46.9 (benzyl-CHH), 71.5 (C_1 & C_3), 123.6 (Ar-C), 125.8 (Ar-C), 126.7 (Ar-C), 128.0 (Ar-C), 129.5 (Ar-C), 142.6 (Ar-C), 144.8 (Ar-C);

HRMS: calcd for $C_{23}H_{32}N$ $[MH]^+$ 322.2589, found 322.2571. The obtained spectroscopic data was in agreement with that previously reported.[35]

Three other compounds were also isolated from this reaction:

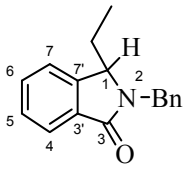
2-Benzyl-3,3-diethylisoindolin-1-one (2.11)



2.11

(Cream coloured solid, 22.0 mg, 22%). Mp 37-39 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.19 (t, J = 7.2 Hz, 6H, 2 x CH_3), 1.86-1.92 (m, 4H, 2 x CH_2), 4.61 (s, 2H, benzyl-CHH), 7.25-7.32 (m, 4H, Ar-H), 7.44 (t, J = 7.2 Hz, 1H, Ar-H), 7.53 (d, J = 7.2 Hz, 3H, Ar-H), 7.89 (d, J = 7.6 Hz, 1H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 7.1 (CH_3), 30.4 (CH_2), 43.0 (benzyl-**C**H_2), 70.7 (C_1), 120.7 (Ar-C), 123.5 (Ar-C), 127.4 (Ar-C), 127.8 (Ar-C), 128.3 (Ar-C), 129.0 (Ar-C), 131.7 (Ar-C), 132.9 (Ar-C), 138.1 (Ar-C), 147.7 (Ar-C), 169.6 (C=O); HRMS: calcd for $C_{19}H_{22}NO$ $[MH]^+$ 280.1700, found 280.1720

2-Benzyl-3-ethylisoindolin-1-one (2.12)



2.12

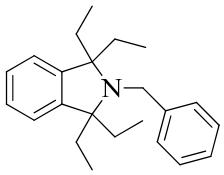
(colourless oil, 3.00 mg, 3%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.54 (t, J = 8.0 Hz, 3H, CH_3), 1.97-2.06 (m, 2H, CH_2), 4.11 (d, J = 15.2 Hz, 1H, alkane-H), 4.45 (t, J = 4 Hz, 1H, benzyl-CHH), 5.44 (d, J = 14.8 Hz, 1H, benzyl-CHH), 7.28-7.37 (m, 6H, Ar-H), 7.48 (t, J = 7.2 Hz, 1H, Ar-H), 7.54 (t, J = 7.2 Hz, 1H, Ar-H), 7.91 (d, J = 7.6 Hz,

1H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 6.3 (CH_3), 22.9 (CH_2), 43.7 (benzyl-**C**HH), 59.0 (C_1), 122.0 (C_7), 123.8 (benzyl-Ar-C), 127.6 (benzyl-Ar-C), 128.0 (benzyl-Ar-C), 128.2 (C_4), 128.7 (C_5 or C_6), 131.4 (C_5 or C_6), 132.7 ($C_{3'}$), 137.2 (benzyl-Ar-C), 144.9 ($C_{7'}$), 168.7 (C=O); HRMS: calcd for $C_{17}H_{18}NO$ $[MH]^+$ 252.1400, found 252.1393. These data were in agreement with those previously reported.[137]

2-Benzyl-3-ethyl-3-hydroxyisoindolin-1-one (2.2)

(White crystalline solid, 13.0 mg, 13%). Data shown above.

2-Benzyl-1,1,3,3-tetraethylisoindoline (1.94) from 2-benzyl-3-ethyl-3-methoxyisoindolin-1-one (2.2).

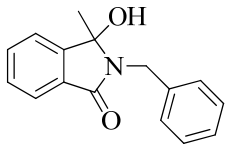


1.94

Ethyl iodide (15.4 mL, 0.191 mol, 6.0 equiv.) was added dropwise to a suspension of pre-dried magnesium turnings (6.11 g, 0.255 mol, 8.0 equiv.) in anhydrous diethyl ether (60 mL). The mixture was stirred at room temperature for three hours and then concentrated by distillation until a temperature of 80 - 90 ºC was reached. The reaction mixture was allowed to cool to 64 ºC and a solution of 2-Benzyl-3-ethyl-3-hydroxyisoindolin-1-one **(2.2)** (8.50 g, 32.0 mmol) in dry toluene (50 mL) was added. Once the addition was completed, the mixture was refluxed at 110 ºC for 3 days. Saturated ammonium chloride solution (80 mL) was then added and the mixture was stirred until all the solids had dissolved. The toluene layer was separated, dried over anhydrous Na_2SO_4 and evaporated to dryness. The dark

brownish purple product obtained was run through a basic alumina column with hexane (2.5 L) to give the title compound **1.94** as a white solid (5.89 g, 57%). mp 72-74 ºC (lit.[147] mp 76 ºC); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.79 (t, J = 7.6 Hz, 12H), 1.53-1.59 (m, 4H), 1.92-1.97 (m, 4H), 4.03 (s, 2H), 7.06-7.09 (m, 2H), 7.21-7.23 (m, 2H), 7.26-7.34 (m, 3H), 7.47 (d, J = 6.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.8, 30.5, 46.9, 71.5, 123.6, 125.8, 126.7, 128.0, 129.5, 142.6, 144.8; HRMS: calcd. for $C_{23}H_{32}N$ $[MH]^+$ 322.2589, found 322.2571. The obtained spectroscopic data was in agreement with that previously reported. [35]

2-Benzyl-3-hydroxy-3-methylisoindolin-1-one (**3.3**)

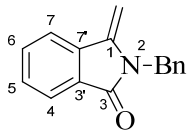


3.3

Methyl iodide (3.30 mL, 53.0 mmol, 2.5 equiv.) was added dropwise to a suspension of pre-dried magnesium turnings (1.27 g, 53.0 mmol, 2.5 equiv.) in anhydrous diethyl ether (about 25 mL). The mixture was stirred for about one hour until all the activity had subsided and then concentrated by distilling off ether until a temperature of 80-90 °C was reached. The reaction mixture was allowed to cool to 64 °C and a solution of *N*-benzylphthalimide (**1.75**) (5.01 g, 21.0 mmol) in dry toluene (20 mL) was added to the Grignard mixture. Once addition was completed, the mixture was stirred for 2 hours at room temperature. Saturated ammonium chloride solution (50 mL) was then added and the mixture stirred until all the solids dissolved. The organic layer was separated and the remaining aqueous layer extracted with chloroform (4x 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated at reduced pressure to give off-white solid (4.98 g, 94%) with a HPLC

purity of 98%. Further purification by recrystallization from hexane/ethyl acetate gave title compound as white coloured crystals (3.35 g, 63%). Mp 164-166 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (s, 3H, CH_3), 3.49 (bs, 1H), 4.48 (d, *J*=15.2 Hz, 1H, benzyl-CHH), 4.60 (d, *J*=15.2 Hz, 1H, benzyl-CHH), 7.23-7.35 (m, 5H, benzyl-Ar-H), 7.46-7.50 (m, 1H, Ar-H), 7.58-7.60 (m, 2H, Ar-H), 7.72 (d, *J* =7.6 Hz, 1H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.8 (CH_3), 41.7 (CH_2), 89.0 (C_1), 121.7 (C_7), 123.5 (Ar-C), 127.2 (Ar-C), 127.8 (Ar-C), 128.5 (Ar-C), 129.5 (Ar-C), 130.0 (Ar-C), 132.6 (Ar-C), 138.5 (Ar-C), 148.3 (Ar-C), 167.4 (C=O); HRMS: calcd for $C_{16}H_{16}NO_2$ $[MH]^+$ 254.1176, found 254.1170; Anal. Calcd. for $C_{16}H_{15}NO_2$: C 75.87, H 5.97, N 5.53, found C 75.87, H 6.02, N 5.47.

2-Benzyl-3-methyleneisoindolin-1-one (**3.1**)

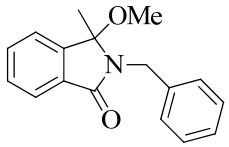


3.1

A solution of 2-benzyl-3-hydroxy-3-methylisoindolin-1-one **3.3** (0.100 g, 0.400 mmol) and triethylamine (80.0 µL, 0.500 mmol, 1.2 equiv.) in dry dichloromethane (5.0 mL) was prepared and purged with argon at room temperature. Triflic chloride (45.0 µL, 0.500 mmol, 1.2 equiv.) was added dropwise to this solution at 0 ºC over a period of 5-10 minutes. The ice bath was removed and the mixture was allowed to reach room temperature and then stirred for 30 minutes. The resulting solution was washed with 2 M HCl (2×40 mL), saturated $NaHCO_3$ solution (2×40 mL) and saturated brine solution (2×40 mL). The combined layers were dried over anhydrous Na_2SO_4 and evaporated at reduced pressure to give a cream colored solid (47.0 mg, 50%) which was purified by recrystallization (ethanol) to give the title compound as white coloured needles (40.0 mg, 42.5%). mp 116-118 °C (lit.[148] 118-119 °C);

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.82 (d, *J*=2.4 Hz, 1H, exocyclic-H), 5.03 (s, 2H, benzyl-CHH), 5.17 (d, *J*=2.4 Hz, 1H, exocyclic-H), 7.27-7.35 (m, 5H, benzyl-Ar-H), 7.54 (t, *J* =7.2 Hz, 1H, Ar-H), 7.60 (t, *J* =7.6 Hz, 1H, Ar-H), 7.69 (d, *J* =7.2 Hz, 1H, Ar-H), 7.91 (d, *J* =7.2 Hz, 1H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 43.2 (benzyl-**C**HH), 90.1 (C_1), 119.9 (exocyclic-alkene-**C**), 123.4 (Ar-C), 127.1 (Ar-C), 127.4 (Ar-C), 128.7 (Ar-C), 129.2 (Ar-C), 129.5 (Ar-C), 132.0 (Ar-C), 136.4 (Ar-C), 136.8 (Ar-C), 141.5 (Ar-C), 167.3 (C=O); HRMS: calcd. for $C_{16}H_{14}NO$ $[MH]^+$ 236.1070, found 236.1079. These data were in agreement with those reported previously by Kundu.[148]

2-Benzyl-3-methyl-3-methoxyisoindolin-1-one (**3.4b**)

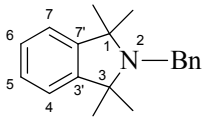


3.4b

Finely ground sodium hydroxide (3.16 g, 80.0 mmol, 4.0 equiv.) was added to a solution of 2-benzyl-3-hydroxy-3-methylisoindolin-1-one **3.3** (5.02 g, 20.0 mmol) in dry THF (25 mL) under an atmosphere of argon. Iodomethane (4.92 mL, 80.0 mmol, 4.0 equiv.) was added dropwise and the mixture was stirred for 7 hours at room temperature. After evaporating to dryness, the obtained residue was dissolved in CH_2Cl_2 (35 mL) and washed with deionized water (2×25 mL). The CH_2Cl_2 layer was dried over anhydrous Na_2SO_4 and concentrated at reduced pressure. The resulting residue (5.01 g, 95%) was purified by recrystallization (hexane) to give title compound as white crystals (4.94 g, 94%). Mp 54-56 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.56 (s, 3H, CH_3), 2.64 (s, 3H, OCH_3), 4.42 (d, *J* =15.2 Hz, 1H, benzyl-

CHH), 4.91 (d, *J* =15.2 Hz, 1H, benzyl-CHH), 7.25-7.34 (m, 3H, Ar-H), 7.44-7.47 (m, 3H, Ar-H), 7.54 (td, *J* =0.8 and 7.6 Hz, 1H, Ar-H), 7.61 (td, *J* =1.2 and 7.2, 1H, Ar-H), 7.90 (d, *J* =7.6 Hz, 1H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.9 (CH_3), 41.8 (benzyl-CHH), 50.2 (OCH_3), 92.9 (Ar-C), 121.9 (Ar-C), 123.6 (Ar-C), 127.2 (Ar-C), 128.3 (Ar-C), 128.4 (Ar-C), 129.7 (Ar-C), 132.0 (Ar-C), 132.3 (Ar-C), 138.2 (Ar-C), 144.6 (Ar-C), 167.8 (C=O); HRMS: calcd. For $C_{17}H_{18}NO_2$ $[MH]^+$ 268.1332, found 268.1345; Anal. Calcd. For $C_{17}H_{17}NO_2$: C 76.38, H 6.41, N 5.24, found C 76.58, H 6.49, N 5.18.

2-Benzyl-1,1,3,3-tetramethylisoindoline (1.76) from *N*-benzylphthalimide (1.75)



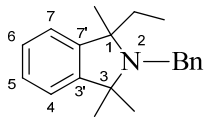
1.76

Methyl iodide (0.80 mL, 13.0 mmol, 6.0 equiv.) was added dropwise to a suspension of pre-dried magnesium turnings (0.450 g, 17.0 mmol, 8.0 equiv.) in anhydrous diethyl ether (25 mL). The mixture was stirred at room temperature for one hour and then concentrated by distillation until a temperature of 80-90 ºC was reached. The reaction mixture was allowed to cool to 64 ºC and a solution of *N*-benzylphthalimide (**1.75**) (0.500 g, 2.00 mmol) in dry toluene (20 mL) was added. Once the addition was completed, the mixture was refluxed at 110 ºC for 4 hours. Saturated ammonium chloride solution (50 mL) was then added and the mixture was stirred until all the solids had dissolved. The toluene layer was separated and evaporated to dryness. The remaining aqueous layer was extracted with chloroform (4×50 mL). The combined chloroform layers were dried over anhydrous Na_2SO_4 and concentrated at reduced pressure. The resulting residues from the toluene and chloroform layers were combined and purified by column chromatography (hexane: ethyl acetate 4:1) to give

the title compound (**1.76**) as a white solid (0.150 g, 27%). Mp 63-65 ºC (lit.[35] mp 63-64 ºC); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.31 (s, 12H, 4 x CH_3), 4.00 (s, 2H, benzyl-CHH), 7.14 (dd, J = 3.2 and 5.6 Hz, 2H, Ar-H), 7.20-7.32 (m, 5H, Ar-H), 7.48 (d, J = 7.6 Hz, 2H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.4 (CH_3), 46.2 (benzyl-CHH), 65.2 (Ar-C), 121.3 (Ar-C), 126.4 (Ar-C), 126.8 (Ar-C), 127.9 (Ar-C), 128.3 (Ar-C), 143.4 (Ar-C), 147.9 (Ar-C); HRMS: calcd for $C_{19}H_{24}N$ $[MH]^+$ 266.1900, found 266.1906. The obtained spectroscopic data was in agreement with that previously reported.[35]

Four other compounds were also isolated from this reaction:

2-Benzyl-1-ethyl-1,3,3-trimethylisoindoline (1.82)



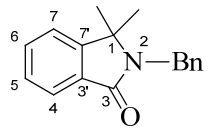
1.82

(colourless oil, 20.0 mg, 3%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.66 (t, J = 7.2 Hz, 3H, CH_3), 1.31 (s, 3H, CH_3), 1.42 (s, 3H, CH_3), 1.43 (s, 3H, CH_3), 1.44-1.55 (m, 1H, ethyl-CHH), 1.59-1.69 (m, 1H, ethyl-CHH), 4.00 (d, J = 2.4 Hz, 2H, benzyl-CHH), 7.07-7.09 (m, 1H, Ar-H), 7.15-7.17 (m, 1H, Ar-H), 7.26-7.30 (m, 3H, Ar-H), 7.35 (tt, J = 2 and 8, 2H, Ar-H), 7.50 (dd, J = 0.8 and 8.4 Hz, 2H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.04 (CH_3), 27.3 (CH_3), 27.8 (CH_3), 29.8 (CH_3), 32.0 (CH_2), 46.9 (benzyl-CHH), 65.4 (Ar-C), 69.0 (Ar-C), 121.3 (Ar-C), 121.7 (Ar-C), 126.6 (Ar-C), 126.70 (Ar-C), 126.71 (Ar-C), 127.9 (Ar-C), 128.9 (Ar-C), 142.8 (Ar-C), 145.3 (Ar-C), 148.7 (Ar-C); HRMS: calcd for $C_{20}H_{26}N$ $[MH]^+$ 280.2100, found 280.2054. The obtained spectroscopic data were slightly different from the previously reported values.[35]

2-Benzyl-3-methyleneisoindolin-1-one (**3.1**)

(Cream crystalline solid, 20.0 mg, 4%). Data shown above.

2-Benzyl-3,3-dimethylisoindolin-1-one (**3.2**)



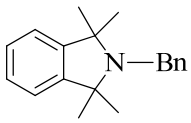
3.2

(Cream solid, 35.0 mg, 7%). Mp 36-39 ºC (lit.[149] mp 38-39 ºC); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.34 (s, 6H, 2 x CH_3), 4.74 (s, 2H, benzyl-CHH), 7.19-7.29 (m, 3H, Ar-H), 7.34-7.37 (m, 3H, Ar-H), 7.44 (dt, J = 1.2 and 7.6 Hz, 1H, Ar-H), 7.53 (dt, J = 1.2 and 7.6 Hz, 1H, Ar-H), 7.88 (d, J = 7.6 Hz, 1H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.3 (CH_3), 42.6 (benzyl-CHH), 63.1 (C_1), 120.6 (Ar-C), 123.8 (Ar-C), 127.1 (Ar-C), 127.7 (Ar-C), 128.0 (Ar-C), 128.4 (Ar-C), 130.4 (Ar-C), 131.7 (Ar-C), 138.7 (Ar-C), 151.9 (Ar-C), 167.9 (C=O); HRMS: calcd for $C_{17}H_{17}NONa$ $[M+Na]^+$ 274.1200, found 274.1201. The obtained spectroscopic data was in agreement with that previously reported.[150]

2-Benzyl-3-hydroxy-3-methylisoindolin-1-one (**3.3**)

(Cream crystalline solid, 0.175 g, 33%). Data shown above.

2-Benzyl-1,1,3,3-tetramethylisoindoline (1.76) from 2-Benzyl-3-hydroxy-3-methylisoindolin-1-one (3.3).



1.76

Methyl iodide (0.75 mL, 12.0 mmol, 6.0 equiv.) was added dropwise to a suspension of pre-dried magnesium turnings (0.380 g, 16.0 mmol, 8.0 equiv.) in anhydrous diethyl ether (25 mL). The mixture was stirred at room temperature for one hour and then concentrated by distillation until a temperature of 80-90 ºC was reached. The reaction mixture was allowed to cool to 64 ºC and a solution of 2-Benzyl-3-hydroxy-3-methylisoindolin-1-one (**3.3**) (0.503 g, 2.00 mmol) in dry toluene (20 mL) was added. Once the addition was completed, the mixture was refluxed at 110 ºC for 72 hours. Saturated ammonium chloride solution (50 mL) was then added and the mixture was stirred until all the solids had dissolved. The toluene layer was separated and evaporated to dryness. The remaining aqueous layer was extracted with chloroform (4×50 mL). The combined chloroform layers were dried over anhydrous Na_2SO_4 and concentrated at reduced pressure. The resulting residues from the toluene and chloroform layers were combined and purified by column chromatography (hexane: ethyl acetate, 4:1) to give the title compound (**1.76**) as a white solid (0.100 g, 20%). Mp 63-65 ºC (lit.[35] mp 63-64 ºC); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.31 (s, 12H), 4.00 (s, 2H), 7.14 (dd, J = 3.2 and 5.6 Hz, 2H), 7.20-7.32 (m, 5H), 7.48 (d, J = 7.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.4, 46.2, 65.2, 121.3, 126.4, 126.8, 127.9, 128.3, 143.4, 147.9; HRMS: calcd for $C_{19}H_{24}N$ $[MH]^+$ 266.1900, found 266.1906. The obtained spectroscopic data was in agreement with that previously reported.[35] Six other compounds were also isolated from this reaction:

2-Benzyl-1-ethyl-1,3,3-trimethylisoindoline (**1.82**)

(Colorless oil, 26.0 mg, 5%). Data shown above.

2-Benzyl-3-methyleneisoindolin-1-one (**3.1**)

(Cream crystalline solid, 49.0 mg, 11%). Data shown above.

2-Benzylphthalimide (**1.75**)

(Cream colored solid, 16.0 mg, 3%). Mp 115-117 ºC (lit.[106] mp 116 ºC); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.84 (s, 2H), 7.26-7.33 (m, 3H), 7.43 (d, J = 7.6 Hz, 2H), 7.70 (dd, J = 3.2 and 5.6 Hz, 2H), 7.84 (dd, J = 3.2 and 5.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 41.6, 123.3, 127.8, 128.6, 128.7, 132.1, 134.0, 136.3, 168.0**;** HRMS: calcd. for $C_{15}H_{11}NO_2Na$ $[M+Na]^+$ 260.0700, found 260.0430. The obtained spectroscopic data were in agreement with the previously reported data.[106]

2-Benzyl-3,3-dimethylisoindolin-1-one (**3.2**)

(Cream solid, 18.0 mg, 3%). Data shown above.

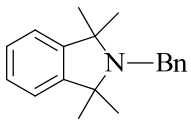
2-Benzyl-3-ethyl-3-hydroxyisoindolin-1-one (**2.2**)

(white crystalline solid, 10.0 mg, 2%). Mp 154-156 ºC (lit.[129] 159 ºC); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.20 (t, J =7.6 Hz, 3H), 1.91-2.11 (m, 2H), 3.54 (s, 1H), 4.37 (d, J =15.2 Hz, 1H), 4.46 (d, J =15.2 Hz, 1H), 7.17-7.26 (m, 3H), 7.38-7.47 (m, 4H), 7.53 (t, J =7.6 Hz, 1H), 7.65 (d, J =7.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 7.47, 29.2, 41.9, 92.5, 121.7, 123.3, 127.3, 128.4, 128.6, 129.5, 131.1, 132.4, 138.2, 146.5, 167.9. The obtained spectroscopic data was in agreement with that previously reported.[140]

2-Benzyl-3-hydroxy-3-methylisoindolin-1-one (**3.3**)

(Cream crystalline solid, 30.0 mg, 6%). Data shown above.

2-Benzyl-1,1,3,3-tetramethylisoindoline (**1.76**) **from 2-Benzyl-3-methyl-3-methoxyisoindolin-1-one** (**3.4b**)



1.76

Methyl iodide (0.70 mL, 11.0 mmol, 6.0 equiv.) was added dropwise to a suspension of pre-dried magnesium turnings (0.360 g, 15.0 mmol, 8.0 equiv.) in anhydrous diethyl ether (25 mL). The mixture was stirred at room temperature for one hour and then concentrated by distillation until a temperature of 80-90 ºC was reached. The reaction mixture was allowed to cool to 64 ºC and a solution of 2-benzyl-3-methyl-3-methoxyisoindolin-1-one **3.4b** (0.500 g, 2.00 mmol) in dry toluene (20 mL) was added. Once the addition was completed, the mixture was refluxed at 110 ºC for 72 hours. Saturated ammonium chloride solution (50 mL) was then added and the mixture was stirred until all the solids had dissolved. The toluene layer was separated and evaporated to dryness. The remaining aqueous layer was extracted with chloroform (4×50 mL). The combined chloroform layers were dried over anhydrous Na_2SO_4 and concentrated at reduced pressure. The resulting residues from the toluene and chloroform layers were combined and purified by column chromatography (hexane: ethyl acetate, 4:1) to give title compound (**1.76**) as a white solid (0.180 g, 36%). Mp 63-65 ºC (lit.[35] mp 63-64 ºC); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.31 (s, 12H), 4.00 (s, 2H), 7.14 (dd, J = 3.2 and 5.6 Hz, 2H), 7.20-7.32 (m, 5H), 7.48 (d, J = 7.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.4, 46.2, 65.2, 121.3,

126.4, 126.8, 127.9, 128.3, 143.4, 147.9; HRMS: calcd. for $C_{19}H_{24}N$ $[MH]^+$ 266.1900, found 266.1906. The obtained spectroscopic data was in agreement with that previously reported.[35]

Six other compounds were also isolated from this reaction:

2-Benzyl-1-ethyl-1,3,3-trimethylisoindoline (**1.82**)

(Colorless oil, 8.00 mg, 1.5%). Data shown above.

2-Benzylphthalimide (**1.75**)

(Cream colored solid, 8.00 mg, 2%). Data shown above.

2-Benzyl-3-methyleneisoindolin-1-one (**3.1**)

(Cream crystalline solid, 5.00 mg, 1%). Data shown above.

2-Benzyl-3,3-dimethylisoindolin-1-one (**3.2**)

(Cream solid, 63.0 mg, 13%); Data shown above.

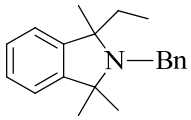
2-Benzyl-3-ethyl-3-hydroxyisoindolin-1-one (**2.2**)

(White crystalline solid, 5.00 mg, 1%). Data shown above.

2-Benzyl-3-hydroxy-3-methylisoindolin-1-one (**3.3**)

(Cream crystalline solid, 28.0 mg, 6%). Data shown above.

2-Benzyl-1-ethyl-,1,3,3-trimethylisoindoline (1.82) from 2-Benzyl-3-methyleneisoindolin-1-one (3.1)



1.82

Methyl iodide (0.95 mL, 15.0 mmol, 6.0 equiv.) was added dropwise to a suspension of pre-dried magnesium turnings (0.50 g, 20.0 mmol, 8.0 equiv.) in anhydrous diethyl ether (25 mL). The mixture was stirred at room temperature for one hour and then concentrated by distillation until a temperature of 80-90 ºC was reached. The reaction mixture was allowed to cool to 64 ºC and a solution of 2-Benzyl-3-methyleneisoindolin-1-one (**3.1**) (0.603 g, 2.00 mmol) in dry toluene (20 mL) was added. Once the addition was completed, the mixture was refluxed at 110 ºC for 72 hours. Saturated ammonium chloride solution (50 mL) was then added and the mixture was stirred until all the solids had dissolved. The toluene layer was separated and evaporated to dryness. The remaining aqueous layer was extracted with chloroform (4×50 mL). The combined chloroform layers were dried over anhydrous Na_2SO_4 and concentrated at reduced pressure. The resulting residues from the toluene and chloroform layers were combined and purified by column chromatography (hexane:ethyl acetate, 4:1) to give title compound (**1.82**) as a colorless oil (35.0 mg, 5%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.66 (t, J = 7.2 Hz, 3H), 1.31 (s, 3H), 1.42 (s, 3H), 1.43 (s, 3H), 1.44-1.55 (m, 1H), 1.59-1.69 (m, 1H), 4.00 (d, J = 2.4 Hz, 2H), 7.07-7.09 (m, 1H), 7.15-7.17 (m, 1H), 7.26-7.30 (m, 3H), 7.35 (tt, J = 2 and 8 Hz, 2H), 7.50 (dd, J = 0.8 and 8.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.04, 27.3, 27.8, 29.8, 32.0, 46.9, 65.4, 69.0, 121.3, 121.7, 126.6, 126.70, 126.71, 127.9, 128.9, 142.8, 145.3, 148.7; HRMS: calcd for $C_{20}H_{26}N$ $[M+H]^+$

280.2100, found 280.2054. The obtained spectroscopic data were slightly different from the previously reported values.[35]

Four other compounds were also isolated from this reaction:

2-Benzylphthalimide (**1.75**)

(Cream colored solid, 38.0 mg, 8%). Data shown above.

***2*-Benzyl-3,3-dimethylisoindolin-1-one** (**3.2**)

(Cream solid, 18.0 mg, 3%). Data shown above.

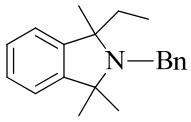
2-Benzyl-3-ethyl-3-hydroxyisoindolin-1-one (**2.2**)

(White crystalline solid, 7.00 mg, 1%). Data shown above.

2-Benzyl-3-hydroxy-3-methylisoindolin-1-one (**3.3**)

(Cream crystalline solid, 32.0 mg, 6%). Data shown above.

2-Benzyl-1-ethyl-,1,3,3-trimethylisoindoline (1.82) from 2-Benzyl-3-ethyl-3-methoxyisoindolin-1-one (2.3b).



1.82

Methyl iodide (1.33 mL, 21.0 mmol, 6.0 equiv.) was added dropwise to a suspension of pre-dried magnesium turnings (0.680 g, 28.0 mmol, 8.0 equiv.) in anhydrous diethyl ether (25 mL). The mixture was stirred at room temperature for one hour and then concentrated by distillation until a temperature of 80-90 ºC was reached. The reaction mixture was allowed to cool to 64 ºC and a solution of 2-benzyl-3-ethyl-3-

methoxyisoindolin-1-one (**2.3b**) (1.05 g, 4.00 mmol) in dry toluene (20 mL) was added. Once the addition was completed, the mixture was refluxed at 110 ºC for 72 hours. Saturated ammonium chloride solution (50 mL) was then added and the mixture was stirred until all the solids had dissolved. The toluene layer was separated and evaporated to dryness. The remaining aqueous layer was extracted with chloroform (4×50 mL). The combined chloroform layers were dried over anhydrous Na_2SO_4 and concentrated at reduced pressure. The resulting residues from the toluene and chloroform layers were combined and purified by column chromatography (hexane:ethyl acetate, 4:1) to give title compound (**1.82**) as a colorless oil (0.290 g, 29%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.66 (t, J = 7.2 Hz, 3H), 1.31 (s, 3H), 1.42 (s, 3H), 1.43 (s, 3H), 1.44-1.55 (m, 1H), 1.59-1.69 (m, 1H), 4.00 (d, J = 2.4 Hz, 2H), 7.07-7.09 (m, 1H), 7.15-7.17 (m, 1H), 7.26-7.30 (m, 3H), 7.35 (tt, J = 2 and 8, 2H), 7.50 (dd, J = 0.8 and 8.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.04, 27.3, 27.8, 29.8, 32.0, 46.9, 65.4, 69.0, 121.3, 121.7, 126.6, 126.70, 126.71, 127.9, 128.9, 142.8, 145.3, 148.7; HRMS: calcd for $C_{20}H_{26}N$ $[MH]^+$ 280.2100, found 280.2054. The obtained spectroscopic data were slightly different from the previously reported values.[35]

Five other compounds were also isolated from this reaction:

2-Benzylphthalimide (**1.75**)

(Cream colored solid, 68.0 mg, 8%). Data shown above.

2-Benzyl-3-methyleneisoindolin-1-one (**3.1**)

(Cream crystalline solid, 23.0 mg, 3%). Data shown above.

2-Benzyl-3,3-dimethylisoindolin-1-one (**3.2**)

(Cream solid, 35.0 mg, 4%). Data shown above.

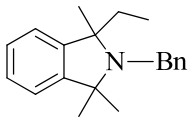
2-Benzyl-3-ethyl-3-hydroxyisoindolin-1-one (**2.2**)

(White crystalline solid, 90.0 mg, 11%). Data shown above.

2-Benzyl-3-hydroxy-3-methylisoindolin-1-one (**3.3**)

(Cream crystalline solid, 50.0 mg, 6%). Data shown above.

2-Benzyl-1-ethyl-,1,3,3-trimethylisoindoline (1.82) from 2-Benzyl-3-ethyl-3-hydroxyisoindolin-1-one (2.2).



1.82

Methyl iodide (0.30 mL, 4.00 mmol, 6.0 equiv.) was added dropwise to a suspension of pre-dried magnesium turnings (0.140 g, 6.00 mmol, 8.0 equiv.) in anhydrous diethyl ether (11 mL). The mixture was stirred at room temperature for one hour and then concentrated by distillation until a temperature of 80-90 ºC was reached. The reaction mixture was allowed to cool to 64 ºC and a solution of 2-benzyl-3-ethyl-3-hydroxyisoindolin-1-one (**2.2**) (0.203 g, 0.750 mmol) in dry toluene (15 mL) was added. Once the addition was completed, the mixture was refluxed at 110 ºC for 72 hours. Saturated ammonium chloride solution (50 mL) was then added and the mixture was stirred until all the solids had dissolved. The toluene layer was separated and evaporated to dryness. The remaining aqueous layer was extracted with chloroform (4×50 mL). The combined chloroform layers were dried over anhydrous Na_2SO_4 and concentrated at reduced pressure. The resulting residues from the

toluene and chloroform layers were combined and purified by column chromatography (hexane:ethyl acetate, 4:1) to give title compound (**1.82**) as a colourless oil (63.0 mg, 30%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.66 (t, J = 7.2 Hz, 3H), 1.31 (s, 3H), 1.42 (s, 3H), 1.43 (s, 3H), 1.44-1.55 (m, 1H), 1.59-1.69 (m, 1H), 4.00 (d, J = 2.4 Hz, 2H), 7.07-7.09 (m, 1H), 7.15-7.17 (m, 1H), 7.26-7.30 (m, 3H), 7.35 (tt, J = 2 and 8, 2H), 7.50 (dd, J = 0.8 and 8.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.04, 27.3, 27.8, 29.8, 32.0, 46.9, 65.4, 69.0, 121.3, 121.7, 126.6, 126.70, 126.71, 127.9, 128.9, 142.8, 145.3, 148.7; HRMS: calcd for $C_{20}H_{26}N$ $[MH]^+$ 280.2100, found 280.2054. The obtained spectroscopic data were slightly different from the previously reported values.[35]

Four other compounds were also isolated from this reaction:

2-Benzylphthalimide (**1.75**)

(Cream colored solid, 5.00 mg, 3%). Data shown above.

2-Benzyl-3,3-dimethylisoindolin-1-one (**3.2**)

(Cream solid, 8.00 mg, 4%). Data shown above.

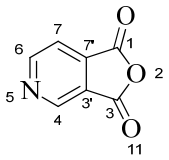
2-Benzyl-3-ethyl-3-hydroxyisoindolin-1-one (**2.2**)

(White crystalline solid, 20.0 mg, 10%). Data shown above.

2-Benzyl-3-hydroxy-3-methylisoindolin-1-one (**3.3**)

(Cream crystalline solid, 6.00 mg, 3%). Data shown above.

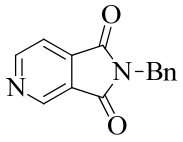
Pyridine-3,4- dicarboxylic anhydride (1.108)



1.108

A mixture of pyridine-3,4-dicarboxylic acid (15.0 g, 90.0 mmol) and acetic anhydride (60 ml, 0.635 mol, 7.0 equiv.) was refluxed for 40 minutes at 140 °C. The excess acetic anhydride was distilled off under high vacuum at 65 °C. The resulting brown colored crude (13.2 g, 98%) was purified by sublimation and white crystals were obtained (12.6 g, 94%). Mp 74-76 °C (lit.[147b] 75-76 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94-7.96 (dd, *J* = 1.2 and 4.8 Hz, 1H, pyr-H), 9.24 (d, *J* = 5.2 Hz, 1H, pyr-H), 9.38 (d, *J* = 0.8 Hz, 1H, pyr-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 118.6 (pyr-Ar-C), 125.3 (pyr-Ar-C), 138.8 (pyr-Ar-C), 147.6 (pyr-Ar-C), 156.8 (pyr-Ar-C), 161.15 (C=O), 161.28 (C=O). These ^{1}H NMR data were in agreement with those reported previously by Mayor and Wentrup.[147b]

***N*-Benzylcinchomeronic imide (1.109)**



1.109

A mixture of pyridine-3,4-dicarboxylic anhydride (12.6 g, 84.0 mmol), acetic acid (65 ml) and benzylamine (18 ml, 0.170 mol, 2.0 equiv.) was refluxed at 120 °C for one hour. The hot reaction mixture was then poured in to ice/H_2O (500 ml) and stirred. The resulting precipitate was filtered and recrystallised from 96% ethanol. The resulting needles like crystals (18.9 g, 95%) were off-white in color. Mp 114-115 °C (lit.[151] 116-117 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.88 (s, 2H,

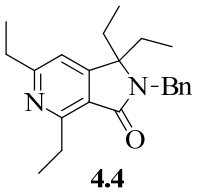
benzyl-CHH), 7.28-7.36 (m, 3H, Ar-H), 7.43-7.45 (m, 2H, Ar-H), 7.76 (dd, *J* = 0.8 and 4.4 Hz, 1H, pyr-H), 9.06 (d, *J* = 4.8 Hz, 1H, pyr-H), 9.16 (d, *J* = 0.8 Hz, 1H, pyr-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 42.0 (benzyl-**C**HH), 116.9 (Ar-C), 125.9 (Ar-C), 128.2 (Ar-C), 128.76 (Ar-C), 128.81 (Ar-C), 135.7 (Ar-C), 139.5 (Ar-C), 144.8 (Ar-C), 155.7 (Ar-C), 166.5 (C=O), 166.9 (C=O); HRMS: calcd for $C_{14}H_{10}N_2O_2$ $[M]^+$ 238.0700, found 238.1000.

Attempted synthesis of 2-benzyl-1,1,3,3-tetraethyl-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine (4.1a) from *N*-benzylcinchomeronic imide (1.109).

Ethyl iodide (0.67 mL, 8.00 mmol, 4.0 equiv.) was added dropwise to a suspension of pre-dried magnesium turnings (0.306 g, 13.0 mmol, 6.0 equiv.) in anhydrous diethyl ether (15 mL). The mixture was stirred at room temperature for one hour and then concentrated by distillation until a temperature of 80-90 ºC was reached. The reaction mixture was allowed to cool to 64 ºC and a solution of *N*-benzylcinchomeronic imide **(1.109)** (0.503 g, 2.00 mmol) in dry toluene (20 mL) was added. Once the addition was completed, the mixture was refluxed at 110 ºC for five hours. Saturated ammonium chloride solution (50 mL) was then added and the mixture was stirred until all the solids had dissolved. The toluene layer was separated and evaporated to dryness. The remaining aqueous layer was extracted with chloroform (4×50 mL). After the first extraction of the aqueous layer by chloroform, aqueous layer was basified by sodium carbonate solution and extracted to chloroform again. The combined chloroform layers were dried over anhydrous Na_2SO_4 and concentrated at reduced pressure. The resulting residues from the toluene and

chloroform layers were combined (0.46 g) and run through a silica column with hexane:ethyl acetate 3:2 to isolate five compounds as follows;

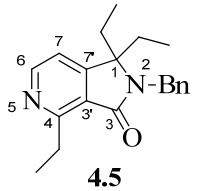
2-Benzyl-1,1,4,6-tetraethyl-1*H*-pyrrolo[3,4-*c*]pyridine-3(2*H*)-one (4.4)



4.4

(White crude, 17.0 mg, 2%). Mp 105-107.5 ºC**;** ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.79 (t, J = 7.2 Hz, 6H, 2 x CH_3), 1.32-1.40 (m, 6H, 2 x CH_3), 1.80-1.92 (m, 4H, 2 x ethyl-CH_2), 2.90 (q, J = 7.6, 2H, pyr-ethyl-CH_2), 3.37 (q, J = 7.6, 2H, pyr-ethyl-CH_2), 4.58 (s, 2H, benzyl-CHH), 6.89 (s, 1H, pyr-H), 7.30-7.34 (m, 3H, benzyl-Ar-H), 7.52 (d, J = 6.8 Hz, 2H, benzyl-Ar-H); HRMS: calcd for $C_{22}H_{29}N_2O$ $[MH]^+$ 337.2300, found 337.2000.

2-Benzyl-1,1,4-triethyl-1*H*-pyrrolo[3,4-*c*]pyridin-3(2*H*)-one (4.5)

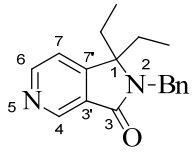


4.5

(White colored solid, 42.0 mg, 6%). Mp 95.5-98 ºC**;** ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.22 (t, J = 7.6 Hz, 6H, 2 x CH_3), 1.40 (t, J = 7.6 Hz, 3H, pyr-CH_3), 1.80-2.00 (m, 4H, 2 x ethyl-CH_2), 3.43 (q, J = 7.6 H z, 2H, pyr-ethyl-CH_2), 4.60 (s, 2H, benzyl-CHH), 7.07 (d, J = 4.8 Hz, 1H, pyr-H), 7.30-7.35 (m, 3H, benzyl-Ar-H), 7.52 (d, J = 7.2 Hz, 2H, benzyl-Ar-H), 8.65 (d, J = 4.8 Hz, 1H, pyr-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 7.1 (CH_3), 13.6 (CH_3), 27.0 (ethyl-**C**H_2), 29.7 (ethyl-**C**H_2), 30.1 (ethyl-**C**H_2), 43.0 (benzyl-**C**HH), 70.0 (C_1), 113.8 (Ar-C), 125.3 (Ar-C), 127.5 (Ar-C),

128.4 (Ar-C), 128.8 (Ar-C), 129.0 (Ar-C), 137.8 (Ar-C), 150.9 (Ar-C), 157.2 (Ar-C), 162.8 (Ar-C), 168.6 (C=O); HRMS: calcd. for $C_{20}H_{25}N_2O$ $[MH]^+$ 309.2000, found 309.1974. The regio-isomerism of the structure was confirmed using a NOESY spectrum.

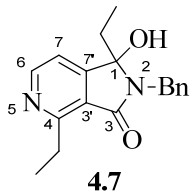
2-Benzyl-1,1-diethyl-1*H*-pyrrolo[3,4-*c*]pyridin-3(2*H*)-one (4.6)



4.6

(White crude, 50.0 mg, 9%). Mp 91-93 ºC**;** ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.22 (t, J = 7.2 Hz, 6H, 2 x CH_3), 1.86-1.97 (m, 4H, 2 x CH_2), 4.62 (s, 2H, benzyl-CHH), 7.28-7.33 (m, 4H, Ar-H), 7.52 (d, J = 7.2 Hz, 2H, Ar-H), 8.78 (d, J = 4.8 Hz, 1H, pyr-H), 9.15 (s, 1H, pyr-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 7.1 (CH_3), 29.7 (ethyl-**C**H_2), 30.0 (ethyl-**C**H_2), 43.0 (benzyl-**C**HH), 71.0 (C_1), 127.7 (Ar-C), 128.5 (Ar-C), 128.80 (Ar-C), 128.81 (Ar-C), 129.0 (Ar-C), 137.5 (Ar-C), 145.9 (Ar-C), 151.8 (Ar-C), 156.4 (Ar-C), 167.9 (C=O); HRMS: calcd for $C_{18}H_{21}N_2O$ $[MH]^+$ 281.1700, found 281.1632.

2-Benzyl-1,4-diethyl-1-hydroxy-1*H*-pyrrolo[3,4-*c*]pyridin-3(2*H*)-one (4.7)

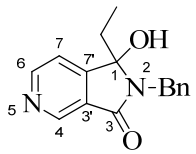


4.7

(Cream colored solid, 0.105 g, 17%). Mp 145-148 ºC**;** ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.39 (t, J = 7.6 Hz, 3H, CH_3), 1.36 (t, J = 7.6 Hz, 3H, CH_3), 2.13 (q, J = 2.8 Hz, 2H, ethyl-CH_2), 3.34 (q, J = 3.6 Hz, 2H, ethyl-CH_2), 4.56 (d, J = 14.8, 1H,

benzyl-CHH), 4.77 (d, *J* = 14.8 Hz, 1H, benzyl-CHH), 7.30-7.35 (m, 4H, Ar-H), 7.50 (d, *J* = 6.8 Hz, 2H, Ar-H), 8.66 (d, *J* = 4.8 Hz, 1H, pyr-Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 7.5 (CH_3), 13.5 (CH_3), 27.0 (ethyl-CH_2), 29.0 (ethyl-CH_2), 42.1 (benzyl-CHH), 91.3 (C_1), 114.5 (Ar-C), 123.3 (Ar-C), 127.6 (Ar-C), 128.6 (Ar-C), 128.7 (Ar-C), 138.0 (Ar-C), 152.1 (Ar-C), 155.5 (Ar-C), 162.7 (Ar-C), 166.7 (C=O); HRMS: calcd. for $C_{18}H_{21}N_2O_2$ $[MH]^+$ 297.1600, found 297.1625.

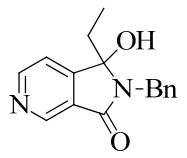
2-Benzyl-1-ethyl-1-hydroxy-1*H*-pyrrolo[3,4-*c*]pyridin-3(2*H*)-one (4.8)



4.8

(Cream colored solid, 0.206 g, 37%). Mp 138-140 ºC**;** ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.30 (t, *J* = 7.6 Hz, 3H, CH_3), 2.00-2.17 (m, 2H, CH_2), 4.55 (d, *J* = 14.8 Hz ,1H, benzyl-CHH), 4.62 (d, *J* = 14.8 Hz, 1H, benzyl-CHH), 4.80 (bs, 1H), 7.26-7.33 (m, 3H, Ar-H), 7.42 (d, *J* = 4.8 Hz, 1H, pyr-H), 7.46 (d, *J* = 7.6 Hz, 2H, Ar-H), 8.47 (d, *J* = 5.2 Hz, 1H, pyr-H), 8.70 (s, 1H, pyr-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 7.4 (CH_3), 29.1 (ethyl-CH_2), 42.1 (benzyl-**C**HH), 92.3 (C_1), 117.1 (Ar-C), 127.2 (Ar-C)**,** 127.6 (Ar-C), 128.5 (Ar-C), 128.9 (Ar-C), 137.6 (Ar-C), 144.3 (Ar-C), 151.9 (Ar-C), 155.7 (Ar-C), 165.8 (C=O); HRMS: calcd for $C_{16}H_{17}N_2O_2$ $[MH]^+$ 269.1300; found 269.1269; Anal. Calcd. for $C_{16}H_{16}N_2O_2$: C 71.62, H 6.01, N 10.44, Found C 71.69, H 6.13, N 10.32. The regio-isomerism of the structure was confirmed using an HMBC spectrum.

2-Benzyl-1-ethyl-1-hydroxy-1*H*-pyrrolo[3,4-*c*]pyridin-3(2*H*)-one (4.8).



4.8

Ethyl iodide (0.85 mL, 10.0 mmol, 2.5 equiv.) was added dropwise to a suspension of pre-dried magnesium turnings (0.250 g, 10.0 mmol, 2.5 equiv.) in anhydrous diethyl ether (15 mL). The mixture was stirred at room temperature for one hour until all the activity had subsided and was then concentrated by distillation until a temperature of 80-90ºC was reached. The reaction mixture was allowed to cool to 64 °C and a solution of *N*-benzylcinchomeronic imide **1.109** (1.00 g, 4.00 mmol) in dry toluene (20 mL) was added. Once addition was completed, the mixture was stirred for 2 hours at room temperature. Saturated ammonium chloride solution (50 mL) was added and the reaction mixture was stirred until all the solids dissolved. The organic layer was separated and the remaining aqueous layer extracted with chloroform (4×50 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated at reduced pressure to give brown colored solid. Purification by column chromatography (hexane:ethyl acetate, 1:1) and recrystallization from hexane/ethyl acetate gave the title compound as white colored crystals (0.480 g, 43%). Mp 138-140 ºC**;** ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.30 (t, J = 7.6 Hz, 3H), 2.00-2.17 (m, 2H), 4.55 (d, J = 14.8 Hz ,1H), 4.62 (d, J = 14.8 Hz, 1H), 4.80 (bs, 1H), 7.26-7.33 (m, 3H), 7.42 (d, J = 4.8 Hz, 1H), 7.46 (d, J = 7.6 Hz, 2H), 8.47 (d, J = 5.2 Hz, 1H), 8.70 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 7.4, 29.1, 42.1, 92.3, 117.1, 127.2**,** 127.6, 128.5, 128.9, 137.6, 144.3, 151.9, 155.7, 165.8; HRMS: calcd. for $C_{16}H_{17}N_2O_2$ $[MH]^+$ 269.1300; found 269.1269; Anal. Calcd. for $C_{16}H_{16}N_2O_2$: C 71.62, H 6.01, N

10.44, Found C 71.69, H 6.13, N 10.32. The regio-isomerism of the structure was confirmed using an HMBC spectrum.

Attempted synthesis of 2-benzyl-1,1,3,3-tetraethyl-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine (4.1a) from 2-benzyl-1-ethyl-1-hydroxy-1*H*-pyrrolo[3,4-*c*]pyridin-3(2*H*)-one (4.8).

Ethyl iodide (0.75 mL, 9.00 mmol, 4.0 equiv.) was added dropwise to a suspension of pre-dried magnesium turnings (0.350 g, 13.0 mmol, 6.0 equiv.) in anhydrous diethyl ether (15 mL). The mixture was stirred at room temperature for one hour and then concentrated by distillation until a temperature of 80-90 ºC was reached. The reaction mixture was allowed to cool to 64 ºC and a solution of **4.8** (0.580 g, 2.00 mmol) in dry toluene (20 mL) was added. Once the addition was completed, the mixture was refluxed at 110 ºC for five hours. Saturated ammonium chloride solution (50 mL) was then added and the mixture was stirred until all the solids had dissolved. The toluene layer was separated and evaporated to dryness. The remaining aqueous layer was extracted with chloroform (4×50 mL). After the first extraction of the aqueous layer by chloroform, aqueous layer was basified by sodium carbonate solution and extracted to chloroform again. The combined chloroform layers were dried over anhydrous Na_2SO_4 and concentrated at reduced pressure. The resulting residues from the toluene and chloroform layers were combined (0.560 g) and run through a silica column with hexane:ethyl acetate 3:2 to isolate five compounds as follows;

2-benzyl-1,1,4,6-tetraethyl-1*H*-pyrrolo[3,4-*c*]pyridine-3(2*H*)-one (4.4)

(White crude, 50.0 mg, 7%) Data shown above.

2-benzyl-1,1,4-triethyl-1*H*-pyrrolo[3,4-*c*]pyridin-3(2*H*)-one (4.5)

(White colored solid, 0.112 g, 17%). Data shown above.

2-benzyl-1,1-diethyl-1*H*-pyrrolo[3,4-*c*]pyridin-3(2*H*)-one (4.6)

(White crude, 77.0 mg, 12%). Data shown above.

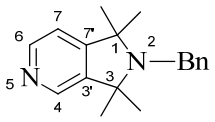
2-benzyl-1,4-diethyl-1-hydroxy-1*H*-pyrrolo[3,4-*c*]pyridin-3(2*H*)-one (4.7)

(Cream colored solid, 0.143 g, 22%). Data shown above.

2-Benzyl-1-ethyl-1-hydroxy-1*H*-pyrrolo[3,4-*c*]pyridin-3(2*H*)-one (4.8)

(Cream colored solid, 0.123 g, 20%). Unreacted starting material.

2-Benzyl-1,1,3,3-tetramethyl-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine (**4.1b**)



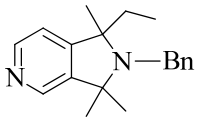
4.1b

Methyl iodide (10.5 mL, 0.168 mol, 8.0 equiv.) was added dropwise to a suspension of pre-dried magnesium turnings (5.04 g, 0.210 mol, 10.0 equiv.) in anhydrous diethyl ether (55 mL). The mixture was stirred at room temperature for one hour and then concentrated by distillation until a temperature of 80-90 ºC was reached. The reaction mixture was allowed to cool to 64 ºC and a solution of *N*-benzylcinchomeronic imide (**1.109**) (3.50 g, 15.0 mmol) in dry toluene (50 mL) was added. Once the addition was completed, the mixture was refluxed at 110 ºC for 72 hours. Saturated ammonium chloride solution (30 mL) was then added and the mixture was stirred for about 1 hour. The toluene layer was then separated and

evaporated to dryness. The remaining brown colored semi-solid layer was extracted to chloroform (5×50 mL). The combined chloroform layers were dried over anhydrous Na_2SO_4 and concentrated at reduced pressure. The resulting residues from the toluene and chloroform layers were combined and purified by column chromatography (hexane:ethyl acetate, 1:3 and 4:1) to give a crude solid. Recrystallization of the crude from hexane gave the title compound (**4.1b**) as a white solid (0.720 g, 18%). Mp 90-92 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.31 (s, 6H, 2 x CH_3), 1.37 (s, 6H, 2 x CH_3), 3.99 (s, 2H, benzyl-CH_2), 7.09 (d, J = 4.8 Hz, 1H, pyr-H_7), 7.23-7.33 (m, 3H, benzyl-Ar-H), 7.46 (d, J = 7.6 Hz, 2H, benzyl-Ar-H), 8.43 (s, 1H, pyr-H_4), 8.49 (d, J = 4.8 Hz, 1H, pyr-H_6); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.8 (CH_3), 28.3 (CH_3), 46.0 (CH_2), 64.5 (C_1 or C_3), 65.3 (C_1 or C_3), 116.7 (C_7), 126.7 (Ar-C), 128.0 (Ar-C), 128.3 (Ar-C), 142.6 (Ar-Cq), 143.6 ($C_{3'}$ or $C_{7'}$), 143.7 (C_4), 148.0 (C_6), 156.8 ($C_{3'}$ or $C_{7'}$); HRMS: calcd. for $C_{18}H_{23}N_2$ $[MH]^+$ 267.1900, found 267.1865. All the carbons in the ^{13}C-NMR spectrum were assigned by running 2D-HSQC and DEPT spectra.

Three other compounds were identified from this reaction:

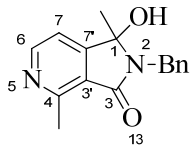
2-Benzyl-1-ethyl-1,3,3-trimethyl-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine (4.14)



4.14

(Colorless oil, 0.220 g, 5%). HRMS: calcd for $C_{19}H_{25}N_2$ $[MH]^+$ 281.2000, found 281.2016. Other characteristic data such as ^{1}H-NMR, ^{13}C-NMR were not definitively assigned due to the presence of some amount of 2-Benzyl-1,1,3,3-tetramethyl-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine **4.1b**, which could not be eliminated by standard purification methods. **(**The ^{1}H-NMR spectrum is shown in the appendix).

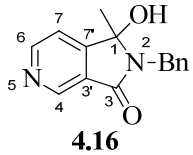
2-Benzyl-1-hydroxy-1,4-dimethyl-1*H*-pyrrolo[3,4-*c*]pyridin-3(2*H*)-one (4.15)



4.15

(Cream colored solid, 0.970 g, 25%). Mp 186-189ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.54 (s, 3H, CH_3), 3.08 (s, 3H, CH_3), 3.76 (bs, 1H, OH), 4.57 (d, J = 15.2 Hz, 1H, benzyl-C**H**H), 4.77 (d, J = 15.2 Hz, 1H, benzyl-CH**H**), 7.23-7.32 (m, 4H, Ar-H), 7.39 (d, J = 7.2 Hz, 2H, Ar-H), 8.46 (d, J = 4.8 Hz, 1H, pyr-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.4 (CH_3), 24.4 (CH_3), 41.8 (CH_2), 87.9 (C_1), 114.5 (Ar-C), 122.9 (Ar-C), 127.4 (Ar-C), 128.0 (Ar-C), 128.6 (Ar-C), 138.1 (Ar-C), 151.8 (Ar-C), 157.1 (Ar-C), 157.5 (Ar-C), 166.3(C_3/C=O); HRMS: calcd for $C_{16}H_{16}N_2NaO_2$ $[M+Na]^+$ 291.1100, found 291.1093. The regio-isomerism of the structure was confirmed using an HMBC spectrum.

2-Benzyl-1-hydroxy-1-methyl-1*H*-pyrrolo[3,4-*c*]pyridin-3(2*H*)-one (4.16)

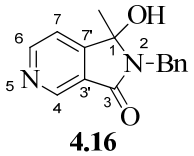


4.16

(Cream colored solid, 1.03 g, 27%). Mp 178-180ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.55 (s, 3H, CH_3), 4.32 (bs, 1H, OH), 4.57 (d, J = 15.2 Hz, 1H, benzyl-C**H**H), 4.70 (d, J = 15.2 Hz, 1H, benzyl-CH**H**), 7.26-7.33 (m, 3H, benzyl-Ar-H), 7.37 (d, J = 7.2 Hz, 2H, benzyl-Ar-H), 7.50 (d, J = 4.8 Hz, 1H, pyr-Ar-H), 8.62 (d, J = 5.2 Hz, 1H, pyr-Ar-H), 8.81 (s, 1H, pyr-Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.4 (CH_3), 41.8 (CH_2), 88.8 (C_1), 116.8 (Ar-C), 126.0 (Ar-C), 127.5 (Ar-C), 128.0 (Ar-C), 128.6 (Ar-C), 137.8 (Ar-C), 144.9 (Ar-C), 152.5 (Ar-C), 156.7 (Ar-C), 165.4 (C_3/C=O); HRMS: calcd for $C_{15}H_{15}N_2O_2$ $[MH]^+$ 255.1100, found 255.1123; Anal. Calcd for

$C_{15}H_{14}N_2O_2$: C 70.85, H 5.55, N 11.02, Found C 70.85, H 5.62, N 10.82. The regio-isomerism of the structure was confirmed using an HMBC spectrum.

2-Benzyl-1-hydroxy-1-methyl-1*H*-pyrrolo[3,4-*c*]pyridin-3(2*H*)-one (4.16).

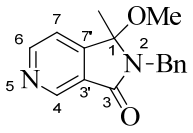


4.16

Methyl iodide (1.31 mL, 21.0 mmol, 2.5 equiv.) was added dropwise to a suspension of pre-dried magnesium turnings (0.503 g, 21.0 mmol, 2.5 equiv.) in anhydrous diethyl ether (20 mL). The mixture was stirred at room temperature for one hour until all the activity had subsided and was then concentrated by distillation until a temperature of 80-90 ºC was reached. The reaction mixture was allowed to cool to 64 °C and a solution of *N*-benzylcinchomeronic imide **1.109** (2.00 g, 8.00 mmol) in dry toluene (20 mL) was added. Once addition was completed, the mixture was stirred for 2 hours at room temperature. Saturated ammonium chloride solution (50 mL) was added and the reaction mixture was stirred until all the solids dissolved. The organic layer was separated and the remaining aqueous layer extracted with chloroform (4×50 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated at reduced pressure to give brown colored solid. Purification by column chromatography (hexane:ethyl acetate, 1:1) and recrystallization from hexane/ethyl acetate gave the title compound as off-white colored crystals (0.820 g, 40%). Mp 178-180 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.55 (s, 3H, CH_3), 4.32 (bs, 1H), 4.57 (d, J = 15.2 Hz, 1H, benzyl-CHH), 4.70 (d, J = 15.2 Hz, 1H, benzyl-CHH), 7.26-7.33 (m, 3H, benzyl-Ar-H), 7.37 (d, J = 7.2 Hz, 2H, benzyl-Ar-H), 7.50 (d, J = 4.8 Hz, 1H, pyr-Ar-H), 8.62 (d, J = 5.2 Hz, 1H, pyr-Ar-H), 8.81 (s, 1H, pyr-Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.4 (CH_3), 41.8 (benzyl-

CHH), 88.8 (C_1), 116.8 (Ar-C), 126.0 (Ar-C), 127.5 (Ar-C), 128.0 (Ar-C), 128.6 (Ar-C), 137.8 (Ar-C), 144.9 (Ar-C), 152.5 (Ar-C), 156.7 (Ar-C), 165.4 (C=O); HRMS: calcd. for $C_{15}H_{15}N_2O_2$ $[M+H]^+$ 255.1100, found 255.1123; Anal. Calcd for $C_{15}H_{14}N_2O_2$: C 70.85, H 5.55, N 11.02, Found C 70.85, H 5.62, N 10.82. The regio-isomerism of the structure was confirmed using an HMBC spectrum.

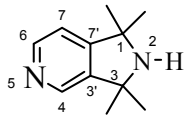
2-Benzyl-1-methoxy-1-methyl-1*H*-pyrrolo[3,4-*c*]pyridin-3(2*H*)-one (4.17b).



4.17b

Finely ground sodium hydroxide (0.104 g, 2.00 mmol, 4.0 equiv.) was added to a solution of **4.16** (0.150 g, 0.600 mmol) in dry THF (25 mL) under an atmosphere of argon. Iodomethane (0.15 mL, 2.00 mmol, 4.0 equiv.) was added dropwise and the mixture was stirred for 24 hours at room temperature. After evaporating to dryness, the obtained residue was dissolved in CH_2Cl_2 (35 mL) and washed with deionized water (2×25 mL). The CH_2Cl_2 layer was dried over anhydrous Na_2SO_4 and concentrated at reduced pressure. The resulting residue (0.135 g, 84%) was purified by recrystallization (hexane) to give title compound as white crystals (0.120 g, 75%). Mp 76-79 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (s, 3H, CH_3), 2.56 (s, 3H, O-CH_3), 4.39 (d, J = 15.2 Hz, 1H, benzyl-CH), 4.85 (d, J = 15.2 Hz, 1H, benzyl-CH), 7.21-7.30 (m, 3H, Ar-H), 7.38-7.40 (m, 3H, Ar-H), 8.82 (d, J = 5.2 Hz, 1H, pyr-Ar-H), 9.11 (s, 1H, pyr-Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.5 (CH_3), 41.9 (benzyl-CH_2), 50.6 (OCH_3), 92.7 (C_1), 116.8 (Ar-C), 127.5 (Ar-C), 128.3 (Ar-C), 128.5 (Ar-C), 137.5 (Ar-C), 145.9 (Ar-C), 152.89 (Ar-C), 152.92 (Ar-C), 166.0 (C_3/C=O); HRMS: calcd for $C_{16}H_{17}N_2O_2$ $[M+H]^+$ 269.1300, found 269.1332.

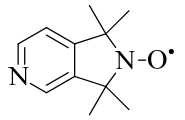
1,1,3,3-Tetramethyl-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine (4.2)



4.2

The starting material 2-Benzyl-1,1,3,3-tetramethyl-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine **4.1b** (80.0 mg, 0.300 mmol) was dissolved in MeOH (15 mL) and the catalyst based on palladium at 10 wt. % on coal was added. Argon was bubbled through the solution for 3-5 minutes to remove any dissolved oxygen in methanol. The reaction vessel was then sealed and hydrogen was introduced to the reaction vessel (*via* a balloon) at atmospheric pressure. The reaction was carried out at room temperature for 3-4 hours with stirring and the complete conversion of the substrate was verified by means of TLC. The reaction mixture was then diluted with methanol and charcoal impurities were removed by celite filtration. The product obtained from MeOH filtrate was further purified by column chromatography (ethylacetate: ethanol 7:1). The titular compound **4.2** was isolated from the column as a colorless oil (50.0 mg, 95%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.40 (s, 6H, CH_3), 1.44 (s, 6H, CH_3), 2.25 (bs, 1H, N-H), 7.03 (d, J = 4.8 Hz, 1H, pyr-H_7), 8.37 (s, 1H, pyr-H_4), 8.43 (d, J = 4.8 Hz, 1H, pyr-H_6); ^{13}C NMR (100 MHz, $CDCl_3$) δ 31.1 (CH_3), 31.8 (CH_3), 62.2 (C_1 or C_3), 62.8 (C_1 or C_3), 116.7 (C_4 or C_6), 143.8 (C_7), 144.3 (C_3' or C_7'), 148.2 (C_4 or C_6), 157.6 (C_3' or C_7'); HRMS: calcd for $C_{11}H_{17}N_2$ $[MH]^+$ 177.1400, found 177.1390. Quetnery carbon peak appeared at 144.3 was identified by a DEPT spectrum while C_4, C_6 and C_7 where identified by 2D-HSQC spectrum.

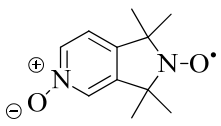
1,1,3,3-Tetramethyl-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridin-2-yloxyl (4.3)



4.3

To a solution of **4.2** (30.0 mg, 0.170 mmol) in methanol (7 mL) and acetonitrile (0.5 mL) was added sodium hydrogen carbonate (11.5 mg, 0.136 mmol, 0.80 equiv.), sodium tungstate dihydrate (2.00 mg, 10.0 µmol, 0.03 equiv.) and finally 30% aqueous hydrogen peroxide (0.10 ml, 0.561 mmol, 3.3 equiv.). The suspension was stiired at room temperature for 24 hours and 0.20 ml of H_2O_2 (30% aqueous) was added again. It was stirred for another 24 hours and a yellow coloured solution was observed. Then it was diluted with distilled water and extracted with dicholoromethane (4x30 mL). The combined organic fractions were dried with anhydrous sodium sulphate and evaporated to give yellow coloured crude, which was purified by column chromatography (ethylacetate: ethanol 7:1). This gave the title nitroxide **4.3** (26.0 mg, 80%). Mp 88-90 ºC**;** HRMS: calcd for $C_{11}H_{16}N_2O$ $[M+H]^+$ 192.1300, found 192.1392; IR (ATR): υ_{max} 3045 (aryl C-H), 2972 and 2929 (alkyl C-H), 1602 and 1574 (quadrant stretch), 1447 (N-O) cm^{-1}; E.p.r. (MeOH): three lines, a_N=1.474 mT, g=2.0058; HPLC purity (MeOH: H2O, 70: 30) 98%.

The other nitroxide **4.3a** isolated from this reaction:

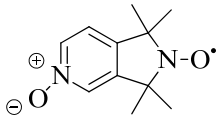


4.3a

(Yellow coloured needles, 6.00 mg, 6%). Mp 172-174 ºC; HRMS: calcd for $C_{11}H_{16}N_2O_2$ $[MH]^+$ 208.1200, found 208.1180; Anal. Calcd. for $C_{11}H_{16}N_2O_2$: C 63.44, H 7.74, N 13.45, Found C 63.59, H 7.34, N 13.58; IR (ATR): υ_{max} 2966 and 2929 (alkyl C-H), 1481 and 1466 (quadrant stretch), 1428 (N-O), 1315 (N-O oxide)

cm^{-1}; E.p.r. (MeOH): three lines, a_N=1.440 mT, g=2.0055; HPLC purity (MeOH: H2O, 70: 30) 100%.

1,1,3,3-Tetramethyl-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridin-5-oxide-2-yloxyl (4.3a)



4.3a

A solution of **4.2** (10.0 mg, 57.0 µmol) in CH_2Cl_2 (6 mL) was cooled to 0 °C and treated with *m*-chloroperbenzoic acid (15.0 mg, 86.0 µmol, 1.5 equiv.). The reaction mixture was stirred at 0 °C for 15 minutes and the cooling bath was removed. Then the reaction mixture was stirred for another 3 hours whilst additional CH_2Cl_2 (4 mL) was added gradually in oreder to dissolve precipitating solids. The reaction mixture was washed with 5M NaOH (20 ml) followed by brine (20 mL), dried with anhydrous Na_2SO_4 and evaporated under reduced pressure. The title nitroxide **4.3a** was resulted as yellow crude (9.60 mg, 81%). Mp 172-174 ºC; HRMS: calcd for $C_{11}H_{16}N_2O_2$ $[MH]^+$ 208.1200, found 208.1180; Anal. Calcd for $C_{11}H_{16}N_2O_2$: C 63.44, H 7.74, N 13.45, Found C 63.59, H 7.34, N 13.58; IR (ATR): υ_{max} 2966 and 2929 (alkyl C-H), 1481 and 1466 (quadrant stretch), 1428 (N-O), 1315 (N-O oxide) cm^{-1}; E.p.r. (MeOH): three lines, a_N=1.440 mT, g=2.0055; HPLC purity (MeOH: H2O 70: 30) 100%.

Preparation of the purple material for MALDI-ToF-MS analysis

Matrix-assisted laser desorption ionoization time-of-flight mass spectrometry (MALDI-ToF-MS) was undertaken on the precipitated purple material (1 mg of the polymer dissolved in 1 mL of DCM) mixed with the matrix, *trans*-2-[3-(4-*tert*-

butylphenyl)-2-methyl-2-propenylidene]malononitrile (20 mg of the matrix was dissolved in 1 mL of DCM) and Na-TFA salt (1 mg of the salt dissolved in 1 mL of DCM) in the ratio 10:10:1 respectively. An aliquot of 4 μL of this sample was then applied to an Ag MALDI probe and allowed to dry at room temperature. The sample was then analysed in both linear and reflection modes.

Preparation of Grignard reaction mixtures for HPLC analysis

1 mg of the Grignard reaction mixture was dissolved in 1 mL of HPLC-grade MeOH. About 0.5 mL (this amount was varied on the visibility of the signals in the HPLC chromatogram; **e.g.:** if signals were not visible, 1 mL sample was used) of this sample was withdrawn and diliuted up to 1 mL with HPLC MeOH. This 1 mL sample was filtered through a filter disk (Whatman 6713-0425 PTFE PolyVENT 4 Venting Filter Discs, 25mm Diameter, 0.2 Micron) and collected to an HPLC vial. This sample was used for HPLC analysis.

Bibliography

1. Perkins, M.J. *Radical Chemistry;* Ellis Horwood Publishing: Hertfordshire, 1994.
2. Hendrickson, J.B.; Cram, D.J.; Hammond, G.S. *Organic Chemistry*; Tosho Printing: Tokyo, 1966.
3. Volodarsky, L.B.; Reznikov, V.A.; Ovcharenko, V.I. *Synthetic Chemistry of Stable Nitroxides;* CRC Press: Florida, 1994.
4. Forrester, A.R.; Hay, J.M.; Thomson, R.H. *Organic Chemistry of Stable Free Radicals;* Academic Press: Michigan, 1968.
5. Kocherginsky, N.; Swartz, H.M. *Nitroxide Spin Labels: Reactions in Biology and Chemistry;* CRC Press: 1995.
6. Rozantsev, E.G.; Sholle, V.D. *Synthesis*, **1971**, *4*, 190-202.
7. Belkin, S.; Mehlhorn, R.J.; Hideg, K.; Hankovsky, O.; Packer, L. *Arch. Biochem. Biophys.* **1987**, *256*, 232-243.
8. Keana, J.F.W.; Pou, S.; Rosen, G.M. *Magn. Reson. Med.* **1987**, *5*, 525-536.
9. Swartz, H.M.; Chen, K.; Pals, M.; Sentjurc, M.; Morse, P.D. *Magn. Reson. Med.* **1986**, *3*, 169-174.
10. Swartz, H.M. *Free Radic. Res. Commun.* **1990**, *9*, 399-405.
11. Chen, K.; Glockner, J.F.; Morse, P.D.; Swartz, H.M. *Biochemistry* **1989**, *28*, 2496-2501.
12. Schafer, F.Q.; Buettner, G.R. *Free Radic. Biol. Med.* **2001**, *30*, 1191-1212.
13. Breuer, E.; Aurich, H.G.; Nielsen, A. *Nitrones, Nitronates and Nitroxides;* John Wiley & Sons: 1989.
14. Chateauneuf, J.; Lusztyk, J.; Ingold, K.U. *J. Org. Chem*. **1988**, *53*, 1629-1632.
15. Beckwith, A.L.J.; Bowry, V.W.; Moad, G. *J. Org. Chem.* **1988**, *53*, 1632-1641.

16. Rodlert, M.; Harth, E.; Rees, I.; Hawker, C.J. *J. Polym. Sci. Part A: Polym. Chem.* **2000**, *38*, 4749-4763.

17. Harth, E.; Hawker, C.J.; Fan, W.; Waymouth, R.M. *Macromolecules* **2001**, *34*, 3856-3862.

18. Braslau, R.; Burrill, L.C.; Siano, M.; Naik, N.; Howden, R.K.; Mahal, L.K. *Macromolecules* **1997**, *30*, 6445-6450.

19. Chalmers, B.A.; Morris, J.A.; Fairfull-Smith, K.E.; Grainger, R.S.; Bottle, S.E. *Chem. Commun.* **2013**, 49, 10382-10384.

20. Halliwell, B.; Gutteridge, J.M.C. *Free Radicals in Biology and Medicine*; Oxford University Press: London, 1999.

21. Wiseman, H.; Halliwell, B.; *Biochem. J.* **1996**, *313*, 17-29.

22. Hess, M.L.; Manson, N.H. *J. Mol. Cell. Cardiol.* **1984**, *16*, 969-985.

23. Fairfull-Smith, K.E.; Brackmann, F.; Bottle, S.E. *Eur. J. Org. Chem.* **2009**, 1902-1915.

24. Krishna, M.C.; Grahame, D.A.; Samuni, A.; Mitchell, J.B.; Russo, A. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 5537-5541.

25. Krishna, M.C.; Russo, A.; Mitchell, J.B.; Goldstein, S.; Dafni, H.; Samuni, A. *J. Biol. Chem.* **1996**, *271*, 26026-26031.

26. Goldstein, S.; Merenyi, G.; Russo, A.; Samuni, A. *J. Am. Chem. Soc.* **2003**, *125*, 789-795.

27. Guichardant, M.; Legarde, M. *Eur. J. Lipid Sci. Technol.* **2009**, *111*, 75-82.

28. Miura, Y.; Utsumi, H.; Hamada, A. *Arch. Biochem. Biophys.* **1993**, *300*, 148-156.

29. Krishna, M.C.; DeGraff, W.; Hankovszky, O.H.; Sar, C.P.; Kalai, T.; Jeko, J.; Russo, A.; Mitchell, J.B.; Hideg, K. *J. Med. Chem.* **1998**, *41*, 3477-3492.

30. Samuni, A.; Godinger, D.; Aronovitch, J.; Russo, A.; Mitchell, J.B. *Biochemistry*

1991, *30*, 555-561.

31. Glebska, J.; Pulaski, L.; Gwozdzinski, K.; Skolimowski, J. *Biometals* **2001**, *14*, 159-170.
32. Krishna, M.C.; Samuni, A.; Taira, J.; Goldstein, S.; Mitchell, J.B.; Russo, A. *J. Biol. Chem.* **1996**, *271*, 26018-26025.
33. a) Thomas, K.; Chalmers, B.A.; Fairfull-Smith, K.E.; Bottle, S.E. *Eur. J. Org. Chem.* **2013**, *5*, 853-857.
    b) Bottle, S.E.; Gillies, D.G.; Hughes, D.L.; Micallef, A.S.; Smirnov, A.I.; Sutcliffe, L.H. *J. Chem. Soc. Perkin Trans. 2* **2000**, 1285–1291.
34. Hageman, T.; Overeen, T. *Makromol. Chem., Rapid Commun.* **1981**, *2*, 719.
35. Griffiths, P.G.; Moad, G.; Rizzardo, E.; Solomon, D.H. *Aust. J. Chem.* **1983**, *36*, 397-401.
36. Busfield, W.K.; Engelhardt, L.M.; Healy, P.C.; Jenkins, I.D.; Thang, S.H.; White, A.H. *Aust. J. Chem.* **1986**, *39*, 357-365.
37. Weber, H.; Grzesiok, A. *Pharmazie* **1995**, *50*, 365-366.
38. Singh, R.J.; Hogg, N.; Mchaourab, H.S.; Kalyanaraman, B. *Biochem. Biophys. Acta* **1994**, *1201*, 437-444.
39. Marx, L.; Rassat, A. *Angew. Chem. Int. Ed.* **2000**, *39*, 4494-4496.
40. Griffiths, P.G.; Rizzardo, E.; Solomon, D.H. *Tetrahedron lett.* **1982**, *23*, 1309-1312.
41. Moad, G.; Solomon, D.H. *The Chemistry of Free Radical Polymerization*; Elsevier Science: Oxford, 1995.
42. Patten, T.E.; Xia, J.; Abernathy, T.; Matyjaszewski, K. *Science* **1996**, *272*, 866-868.
43. Matyjaszewski, K.; Coca, S.; Gaynor, S.G.; Greszta, D.; Patten, T.E.; Wang, J.S.;

Xia, J. *PCT Int. Appl. WO 9718247 A1 19970522* **1997**.

44. Wang, J.S.; Matyjaszewski, K. *J. Am. Chem. Soc.* **1995**, *117*, 5614-5615.
45. Dargaville, T.R.; George, G.A.; Hill, D.J.T.; Whittaker, A.K. *Macromolecules* **2004**, *37*, 360-366.
46. Moad, G.; Chiefari, J.; Kristina, J.; Postma, A.; Mayadunne, R.T.A.; Rizzardo, E.; Thang, S.H. *Polym. Int.* **2000**, *49*, 993-1001.
47. Chiefari, J.; Chong, Y.K.; Ercole, F.; Kristina, J.; Jeffery, J.; Le, T.P.T.; Mayadunne, R.T.A.; Meijs, G.F.; Moad, C.L.; Moad, G.; Rizzardo, E.; Thang, S.H. *Macromolecules* **1998**, *31*, 5559-5562.
48. Destarac, M. *Macromol. React. Eng.* **2010**, *4*, 165.
49. Hawker, C.J.; Bosman, A.W.; Harth, E. *Chem. Rev.* **2001**, *101*, 3661.
50. Cresidio, S.P.; Aldabbagh, F.; Busfield, W.K.; Jenkins, I.D.; Thang, S.H.; Holdsworth, C.Z.; Zetterlund, P.B. *J. Polym. Sci. Part A* **2001**, *39*, 1232-1241.
51. Bartlett, P.D.; Benzing, E.P.; Pincock, R.E. *J. Am. Chem. Soc.* **1960**, *82*, 1762-1768.
52. Skene, W.G.; Belt, S.T.; Connolly, T.J.; Hahn, P.; Scaiano, J.C. *Macromolecules* **1998**, *31*, 9103.
53. Iwao, K.; Sakakibara, K.; Hirota, M. *J. Comput. Chem.* **1998**, *19*, 215.
54. Marsal, P.; Roche, M.; Tordo, P.; de Sainte Claire, P. *J. Phys. Chem.* **1999**, *103*, 2899.
55. Moad, G.; Rizzardo, E. *Macromolecule* **1995**, *28*, 8722.
56. Moad, G.; Rizzardo, E. *Pacific Polymer Conference Preprints*; *Polymer Division, Royal Australian Chemical Institute: Brisbane* **1993**, *3*, 651-652.
57. Kazmaier, P.M.; Moffat, K.A.; Georges, M.K.; Veregin, R.P.N.; Hamer, G.K. *Macromolecules* **1995**, *28*, 1841-1846.

58. Colwell, J.M.; Blinco, J.P.; Hulbert, C.; Fairfull-Smith, K.E.; Bottle, S.E. *Aust. J. Chem.* **2011**, *64*, 426-432.

59. Blinco, J.P.; Fairfull-Smith, K.E.; Morrow, B.J.; Bottle, S.E. *Aust. J. Chem.* **2011**, *64*, 373-389.

60. Likhtenstein, G.I.; Ishii, K.; Nakatsuji, S.I. *Photochem. Photobiol.* **2007**, *83*, 871.

61. Blough, N.V.; Simpson, D.J. *J. Am. Chem. Soc.* **1988**, *110*, 1915-1917.

62. Green, S.A.; Simpson, D.J.; Zhou, G.; Ho, P.S.; Blough, N.V. *J. Am. Chem. Soc.* **1990**, *112*, 7337-7346.

63. Coenjarts, C.; Garcia, O.; Llauger, L.; Palfreyman, J.; Vinette, A.L.; Scaiano, J.C. *J. Am. Chem. Soc.* **2003**, *125*, 620-621.

64. Ivan, M.G.; Scaiano, J.C. *Photochem. Photobiol*. **2003**, *78*, 416-419.

65. Keddie, D.J.; Johnson, T.E.; Arnold, D.P.; Bottle, S.E. *Org. Biomol. Chem.* **2005**, *3*, 2593-2598.

66. Blinco, J.P.; McMurtrie, J.C.; Bottle, S.E. *Eur. J. Org. Chem.* **2007**, *48*, 4638-4641.

67. Blinco, J.P.; George, G.A.; Bottle, S.E. *Polym. Prepr.* **2007**, *48*, 629-630.

68. Fairfull-Smith, K.E.; Bottle, S.E. *Eur. J. Org. Chem.* **2008**, 5391-5400.

69. Micallef, A.S.; Bottle, S.E.; Blinco, J.P.; George, G.A. *ACS Symposium Series* **2008**, *978,* 59-69.

70. Colwell, J.M.; Nikolic, M.A.L.; Bottle, S.E.; George, G.A. *Polym. Degrad. Stab.***2013**, *98*, 2436-2444.

71. a) Stone, T.J.; Buckman, T.; Nordio, P.L.; McConnell, H.M. *Proc. Natl. Acad. Sci. U.S.A.* **1965**, *54*, 1010-1017.

b) Griffith, O.H.; McConnell, H.M. *Proc. Natl. Acad. Sci. U.S.A.* **1966**, *55*, 8-11.

72. Gophane, D.B.; Sigurdsson, S.T. *Chem. Commun.* **2013**, *49*, 999-1001.

73. Fanucci, G.E.; Cafiso, D.S. *Curr. Opin. Struct. Biol.* **2006**, *16*, 644-653.

74. Shelke, S.A.; Sigurdsson, S.T. *Eur. J. Org. Chem.* **2012**, 2291-2301.

75. Bottle, S.E.; Gillies, D.G.; Hughes, D.L.; Micallef, A.S.; Smirnov, A.I.; Sutcliffe, L.H. *J. Chem. Soc. Perkin Trans. 2* **2000,** *7*, 1285-1291.

76. Gryn'ova, G.; Barakat, J.M.; Blinco, J.P.; Bottle, S.E.; Coote, M.L. *Chem. Eur. J.* **2012,** *18*, 7582-7593.

77. Wang, L.-Y.; Zhang, Chen-Xi; Liao, Dai-Zheng; Jiang, Zong-Hui; Yan, Shi-Ping *J. Coord. Chem.* **2005**, *58*, 969-973.

78. Meyer, K.H.; Reppe, W. *Berichte der Deutschen Chemischen Gesellschaft* **1921**, *54*, 327-337.

79. Rozantsev, E.G. *Free Nitroxyl Radicals;* Plenum Press: New York, 1970.

80. Brik, M.E. *Heterocycles* **1995**, *41*, 2827.

81. Naik, N.; Braslau, R. *Tetrahedron* **1998**, *54*, 667.

82. Lemaire, M.T. *Pure Appl. Chem.* **2004**, *76*, 277.

83. Rosen, G.M.; Britigan, B.E.; Halpern, H.J.; Pou, S. *Free Radicals: Biology and Detection by Spin Trapping*; Oxford University Press: Oxford, 1999.

84. Berliner, L.J. *Spin Labelling: The Next Millennium*; Plenum Press: New York, 1998.

85. Hideg, K.; Hankovszky, H.O. *Spin Labeling Theory and Applications*; Plenum Press: New York, 1989.

86. Aurich, H.G. *Nitrones, Nitronates and Nitroxides*; John Wiley & Sons: Chichester, 2010.

87. a) Micallef, A.S.; Bott, R.C.; Bottle, S.E.; Smith, G.; White, J.M.; Matsuda, K.; Iwamura, H. *J. Chem. Soc. Perkin Trans. 2* **1999**, *2*, 65-71.

b) Fairfull-Smith, K.E.; Debele, E.A.; Allen. J.P.; Pfrunder, M.C.; McMurtrie,

J.C. *Eur. J. Org. Chem.* **2013**, 4829-4835.

88. Caldararo, M.; Po, R.; Riccci, M. *PCT Int. Appl. WO2006029697* **2006**.

89. Kalai, T.; Balog, M.; Jeko, J.; Hideg, K. *Synthesis* **1999**, *6*, 973-980.

90. Mashraqui, S.; Keehn, P. *Synth. Commun.* **1982**, *12*, 637.

91. Fu, P.P.; Harvey, R.G. *Chem. Rev.* **1978**, *78*, 317.

92. Reid, D.; Bottle, S.E.; Micallef, A.S. *Chem. Commun.* **1998**, *17*, 1907-1908.

93. Hideg, K.; Hankovszky, H.O.; Halasz, H.A. *J. Chem. Soc. Perkin Trans. 1* **1988**, *11*, 2905-2911.

94. Havinga, E.; de Kock, R.J.; Rappoldt, M.P. *Tetrahedron* **1960**, *11*, 276.

95. ten Have, R.; van Leusen, A.M. *Tetrahedron* **1998**, *54*, 1913.

96. Chaumontet, M.; Pccardi, R.; Baudoin, O. *Angew. Chem. Int. Ed.* **2009**, *48*, 179.

97. Kano, S.; Sugino, E.; Sjhibuya, S.; Hibino, S. *J. Org. Chem.* **1981**, *46*, 3856.

98. Kalai, T.; Jeko, J.; Hideg, K. *Synthesis* **2009**, *15*, 2591-2595.

99. Berenyi, S.; Sipos, A.; Szabo, I.; Kalai, T. *Synth. Commun.* **2007**, *37*, 467.

100. Kalai, T.; Szabo, Z.; Jeko, J.; Hideg, K. *Org. Prep. Proced. Int.* **1996**, *28*, 289.

101. Kalai, T.; Balog, M.; Jeko, J.; Hideg, K. *Synthesis* **1998**, *10*, 1476-1482.

102. Zhdanov, R.I. *Bioactive Spin Labels*; Springer-Verlag Publishing: Berlin, 1992.

103. Chiusoli, G.P.; Costa, M.; Zhiming, Z. *Gazz. Chim. Ital.* **1992**, *122*, 441-449.

104. Kopka, I.E.; Fataftah, Z.A.; Rathke, M.W. *J. Org. Chem.* **1980**, *45*, 4616-4622.

105. Heidenbluth, K.; Scheffler, R. *J. Prakt. Chem.* **1964**, 2*3*, 59.

106. Manske, R.H.F. *Organic Synthesis* **1932**, *XII*, 10-11.

107. March, J. *Advanced Organic Chemistry: Reactions, Mechanisms and Structure* (4th *Ed.)*; John Wiley and Sons: New York, 1992.

108. Braslau, R.; Chaplinski, V. *J. Org. Chem.* **1998**, *63*, 9857-9864.

109. Fairfull-Smith, K.E.; Brackmann, F.; Bottle, S.E. *Eur. J. Org. Chem.* **2009**,

1902-1915.

110. Caldararo, M.; Po, R.; Riccci, M. *PCT Int. Appl. WO2007093452* **2007**.

111. Foitzik, R.C.; Bottle, S.E.; White, J.M.; Scammells, P.J. *Aust. J. Chem.* **2008**, *61*, 168-171.

112. Kappe, C.O. *Angew. Chem. Int. Ed.* **2004**, *43*, 6250.

113. Gold, H.; Larhed, M.; Nilsson, P. *Synlett* **2005**, *10*, 1596.

114. Walla, P.; Kappe, C.O. *Chem. Commun.* **2004**, *5*, 564-565.

115. Dressen, M.H.C.L.; van de Kruijs, B.H.P.; Meuldijk, J.; Vekemans, J.A.J.M.; Hulshof, L.A. *Org. Process Res. Dev.* **2007**, *11*, 865.

116. Ashby, E.C.; Laemmle, J.; Neumann, H.M. *Acc. Chem. Res.* **1974**, *7*, 272-280.

117. Hicks, R. *Stable Radicals: Fundamentals and Applied Aspects of Odd-Electron Compounds*; John Wiley & Sons: Chichester (UK), 2010.

118. a) Keana, J.F.; Acarregui, M.J.; Boyle, S.L.M. *J. Am. Chem. Soc.* **1982**, *104*, 827-830.

b) Khlestkin, V.K.; Butakov, V.V.; Grigor'ev, I.A. *Synthesis* **2005**, *20*, 3649-3653.

119. Bobko, A.A.; Efimova, O.V.; Voinov, M.A.; Khramtsov, V.V. *Free Radic. Res.* **2012**, *46*, 1115-1122.

120. a) Vaz, M.G.F.; Pinheiro, M.M.; Stumpf, H.O.; Alcantara, A.F.C.; Golhen, S.; Ouahab, L.; Cabr, O.; Mathoniere, C.; Kahn, O. *Chem. Eur. J.* **1999**, *5*, 1486.

b) Laget, V.; Hornick, C.; Rabu, P.; Drillon, M.; Ziessel, R. *Coord. Chem. Rev.* **1998**, *178*, 1549.

121. Budzelaar, P.H.M. *Eur. J. Inorg. Chem.* **2012**, *3*, 530-534.

122. Jastrzebski, J.T.B.H.; Klerks, J.M.; Vankoten, G.; Vrieze, K. *J. Organomet. Chem.* **1981**, *210*, C49-C53.

123. Kalai, T.; Jeko, J.; Hideg, K. *Synthesis* **2000**, *6*, 831-837.

124. a) Kalai, T.; Balog, M.; Jeko, J.; Hubbell, W.L.; Hideg, K. *Synthesis* **2002**, *16*, 2365-2372.

b) Chiusoli, G.P.; Pallini, L.; Terenghi, G. *Eur. Pat. Appl. EP 92288 A2 19831026* **1983**.

125. Marx, L.; Chiarelli, R.; Guiberteau, T.; Rassat, A. *J. Chem. Soc. Perkin Trans. 1* **2000**, *8*, 1181-1182.

126. Nicolas, J.; Guillaneuf, Y.; Lefay, C.; Bertin, D.; Gigmes, D.; Charleux, B. *Prog. Polym. Sci.* **2013**, *38*, 63-235.

127. Solomon, D.H.; Rizzardo, E.; Cacioli, P. *Eur. Pat. Appl. 135280* **1985**, 102.

128. Chan, K.S.; Li, X.Z.; Lee, S.Y. *Organometallics* **2010**, *29*, 2850-2856.

129. Heidenbluth, V.K.; Tonjes, H.; Sheffler, R. *J. Prakt. Chem.* **1965**, *30*, 204-217.

130. Wolfe, M.S.; Lee, Y.; Bartlett, W.J.; Borcherding, D.R.; Borchardt, R.T. *J. Med. Chem.* **1992**, *35*, 1782-1791.

131. Clayden, J.; Greeves, N.; Warren, S.; Wothers, P. *Organic Chemistry*; Oxford University Press: Oxford, 2001.

132. Marsili, A.; Scartoni, V. *Gazz. Chim. Ital.* **1972**, *102*, 507.

133. Freccero, M.; Fasani, E.; Albini, A. *J. Org. Chem.* **1993**, *58*, 1740-1745.

134. Kemp, S.J.; Hardcastle, I.R.; Ahmed, S.U.; Atatreh, N.A.; Barrett, P.; Endicott, J.A.; Golding, B.T.; Griffin, R.J.; Gruber, J.; Hutton, C.; Kallblad, P.; Kemp, S.J.; Kitching, M.S.; Newell, D.R.; Norbedo, S.; Northen, J.S.; Reid, R.J.; Saravanan, K.; Willems, H.M.G.; Lunec, J. *Bioorg. Med. Chem. Lett.* **2005**, 15, 1515-1520.

135. Gilman, H.; McCracken, R. *J. Am. Chem. Soc.* **1923**, *45*, 2462-2466.

136. Blomberg, C. *Handbook of Grignard Reagents*; CRC Press: New York, 1996.

137. Augner, D.; Gerbino, D.C.; Slavov, N.; Neudorfl, J.M.; Schmalz, H.G. *Org. Lett.* **2011**, *13*, 5374-5377.

138. Ashby, E.C.; Goel, A.B. *J. Am. Chem. Soc.* **1981**, *103*, 4983-4985.

139. Caldararo, M.; Po, R.; Riccci, M.; Schimperna, G.; Cardi, N. *PCT Int. Appl. WO2004078720* **2004**.

140. Jayawardena, V.C.; Fairfull-Smith, K.E.; Bottle, S.E. *Aust. J. Chem.* ***2013***, 66, 619-625.

141. Cao, H.; McNamee, L.; Alper, H. *Org. Lett.* **2008**, *10*, 5281-5284.

142. Hansson, C.; Wickberg, B. *J. Org. Chem.* **1973**, *38*, 3074.

143. Gophane, D.B.; Sigurdsson, S.T. *Chem.Commun.* **2013**, *49*, 999-1001.

144. Fuson, R. C. *J. Am. Chem. Soc.* **1926,** *48*, 2681–2689.

145. Tonjes, H.; Heidenbluth, K.R.; Scheffler, R. *J. Prakt. Chem./Chem.-Ztg* **1964,** *26*, 218-224.

146. Jaroszewska-Manaj, J.; Oszczapowicz, J.; Makulski, W. *J. Chem. Soc. Perkin Trans. 2* **2001**, 1186-1191.

147. a) Krapcho, A.P.; Petry, M.E.; Getahun, Z.; Landi, J.J.; Stallman, J.; Polsenberg, J.F.; Gallagher, C.E.; Maresch, M.J.; Hacker, M.P. *J. Med. Chem.* **1994**, *37*, 828-837.

b) Mayor, C.; Wentrup, C. *J. Am. Chem. Soc.* **1975**, *97*, 7467-7480.

148. Kundu, N.G.; Khan, M.W. *Tetrahedron* **2000**, *56*, 4777-4792.

149. Fabrycy, A.; Kosmider, A. *Roczniki Chemii.* **1973**, *47*, 937-942.

150. He, Z.; Yudin, A.K. *Org. Lett.* **2006**, *8*, 5829-5832.

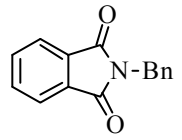
151. Hunter, W.H.; King, J.; Millard, B.J. *Brtt. GB 1086637 19671011* **1967**.

152. Blinco, J.P.; McMurtrie, J.C.; Bottle, S.E. *Eur. J. Org. Chem.* **2007**, *56*, 4638-4641.

Appendices

8.1 ^{1}H-NMR SPECTRA

Figure A1: ^{1}H-NMR spectrum of **1.75** ($CDCl_3$)



1.75

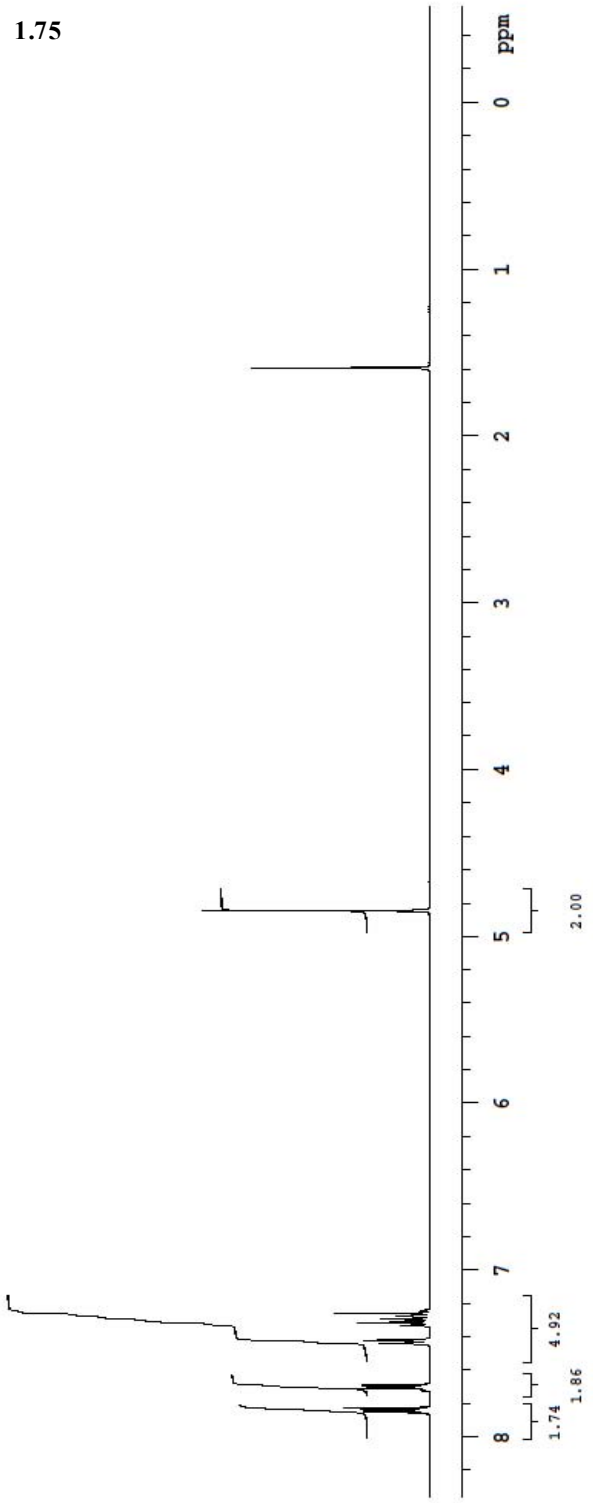
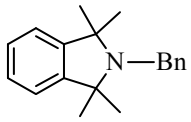


Figure A2: ^{1}H-NMR spectrum of **1.76** ($CDCl_3$)



1.76

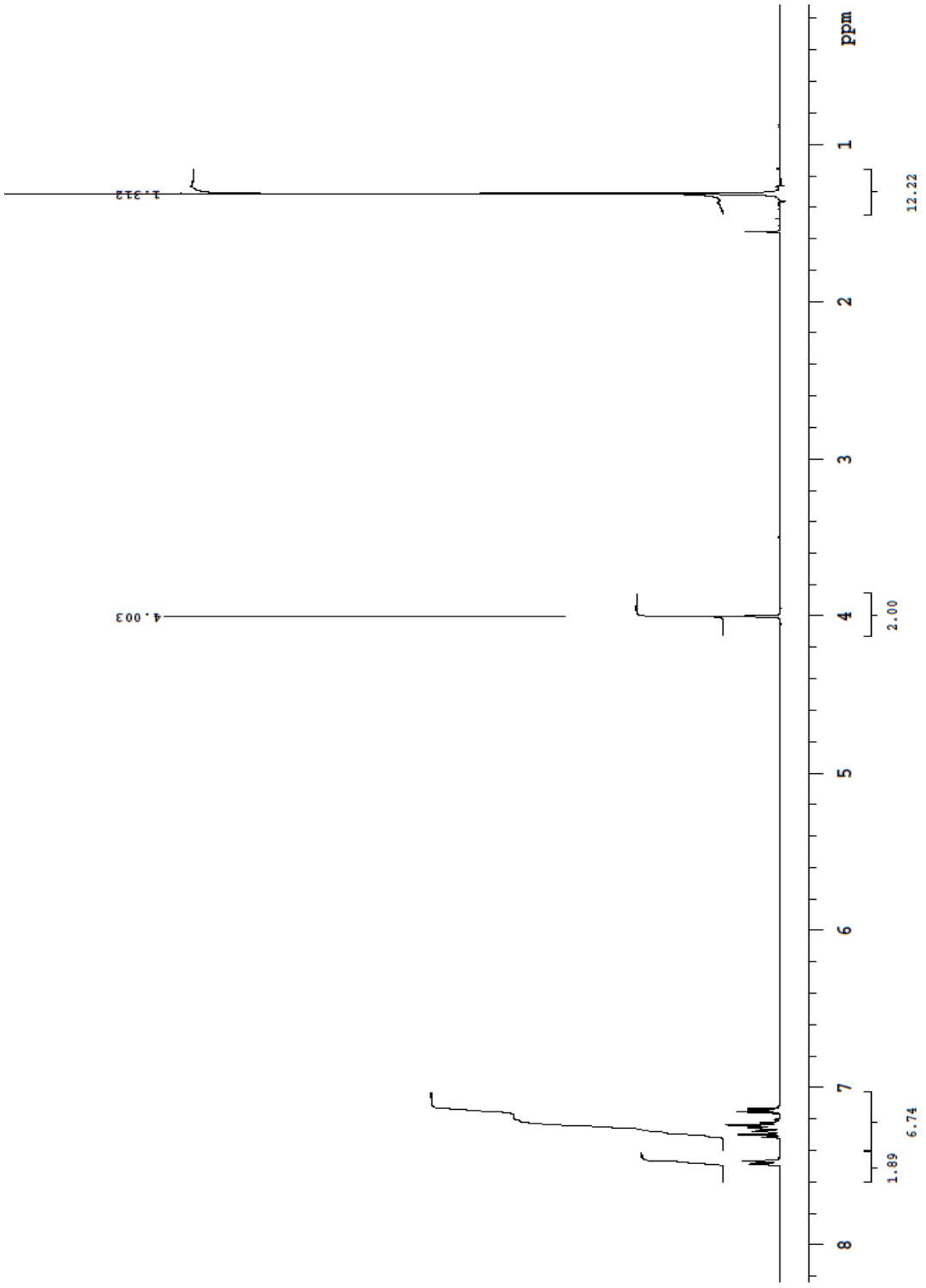
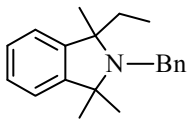


Figure A3: ^{1}H-NMR spectrum of **1.82** ($CDCl_3$)



1.82

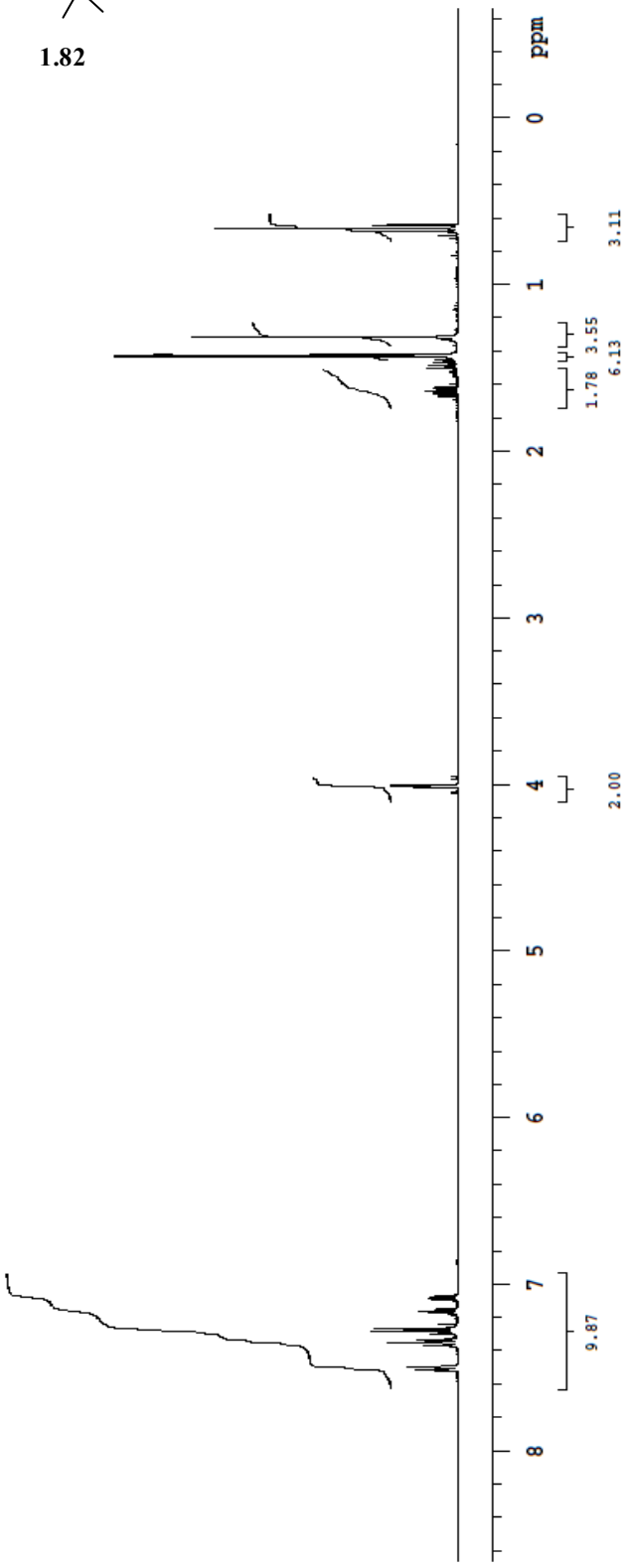
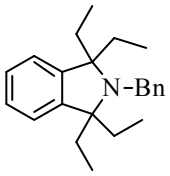


Figure A4: ^{1}H-NMR spectrum of **1.94** ($CDCl_3$)



1.94

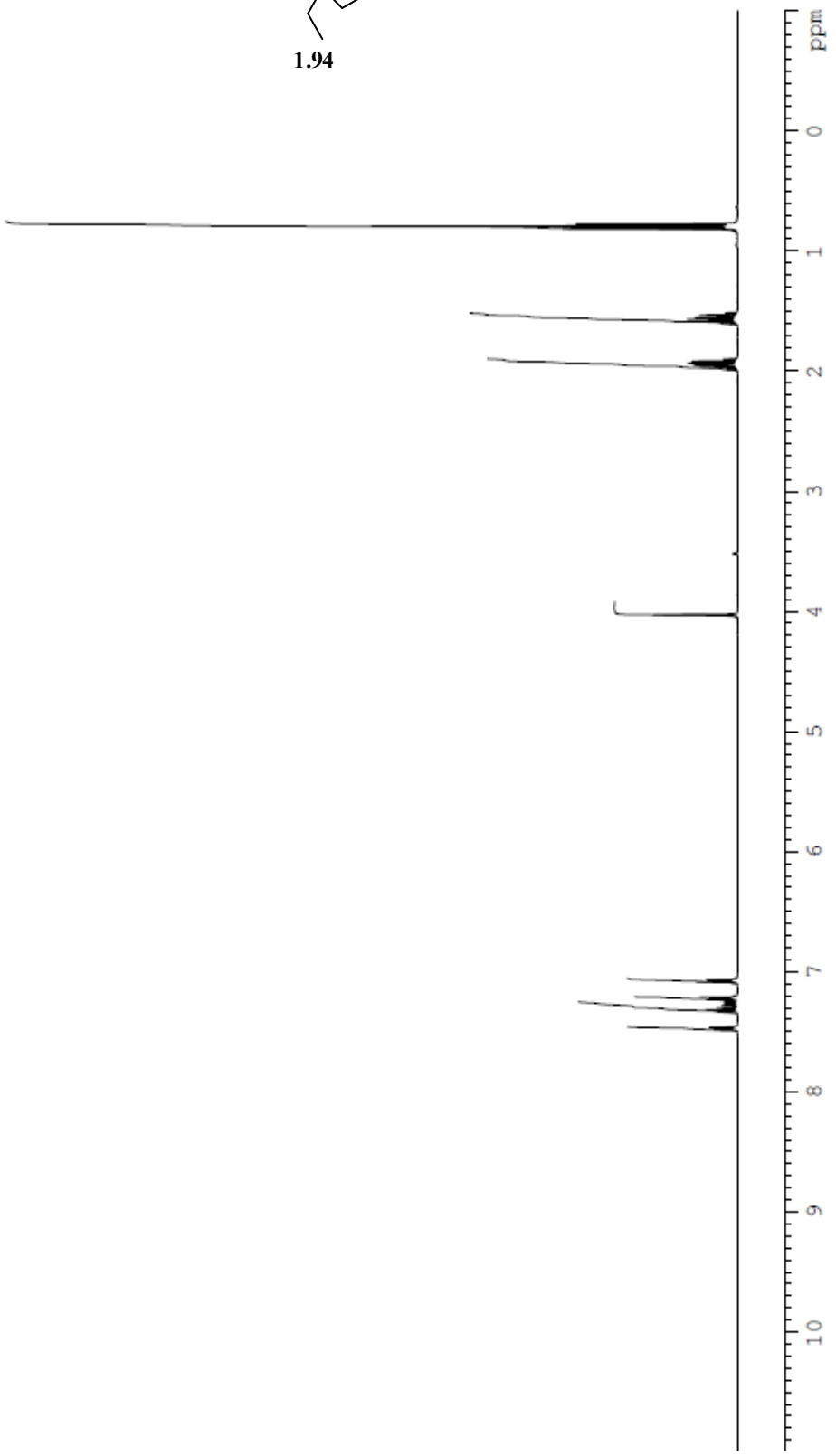


Figure A5: ^{1}H-NMR spectrum of **1.108** ($CDCl_3$)

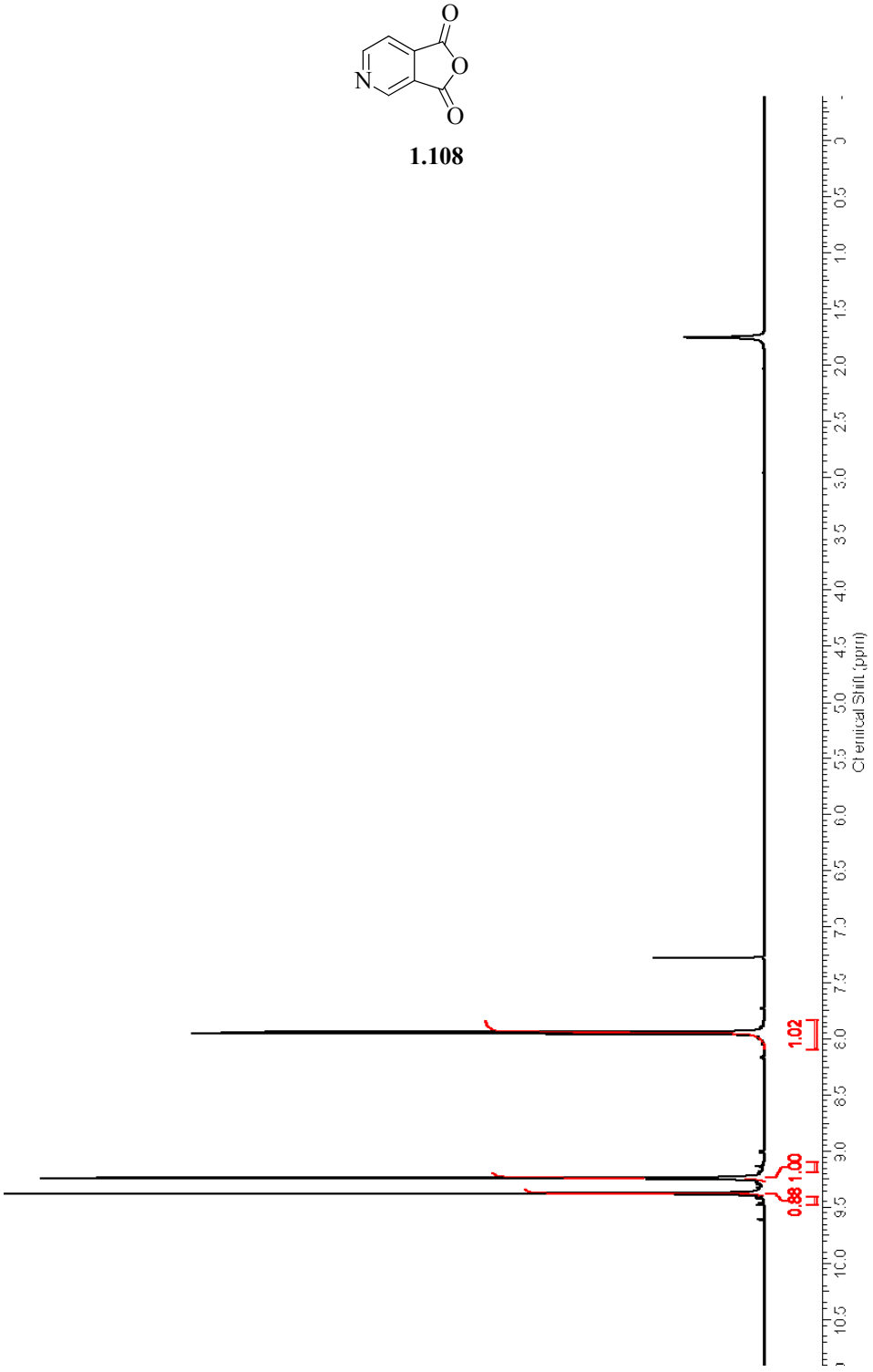


Figure A6: ^{1}H-NMR spectrum of **1.109** ($CDCl_3$)

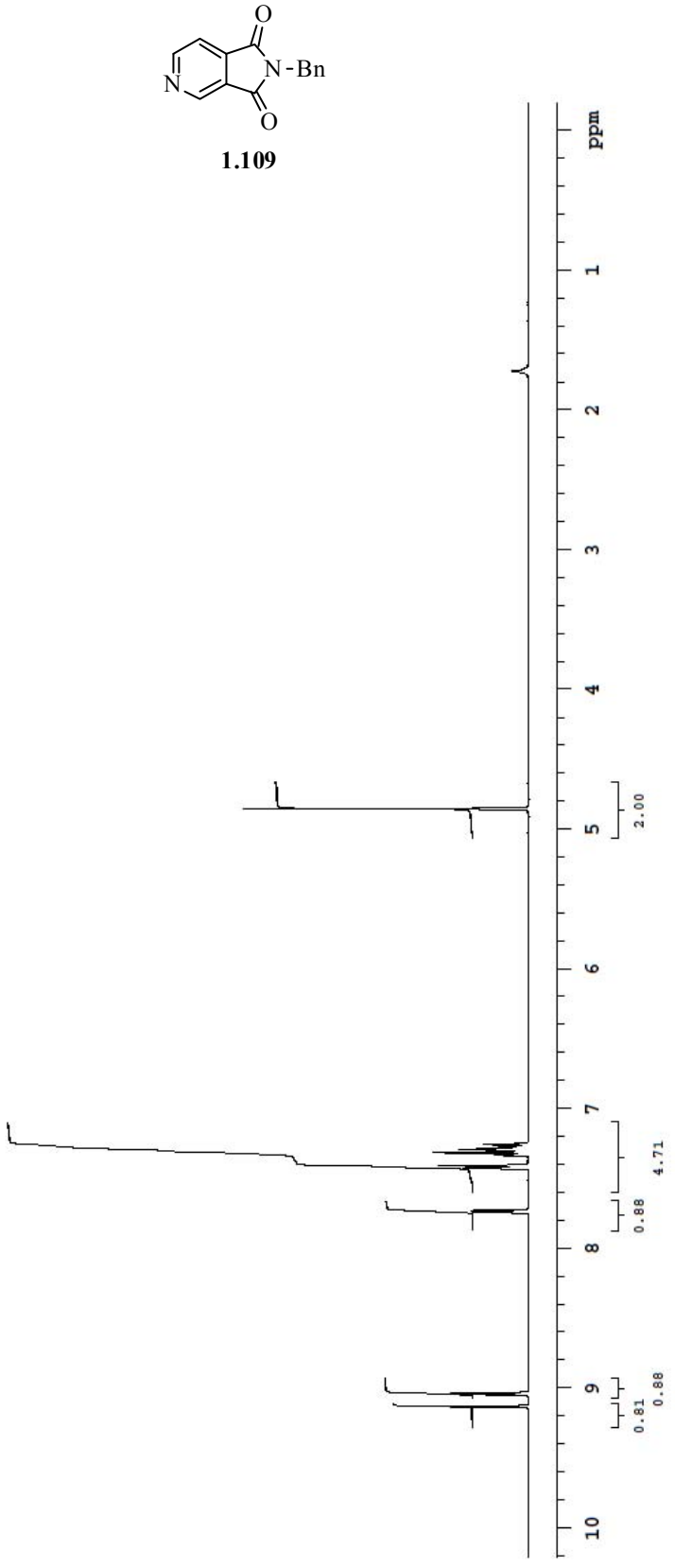
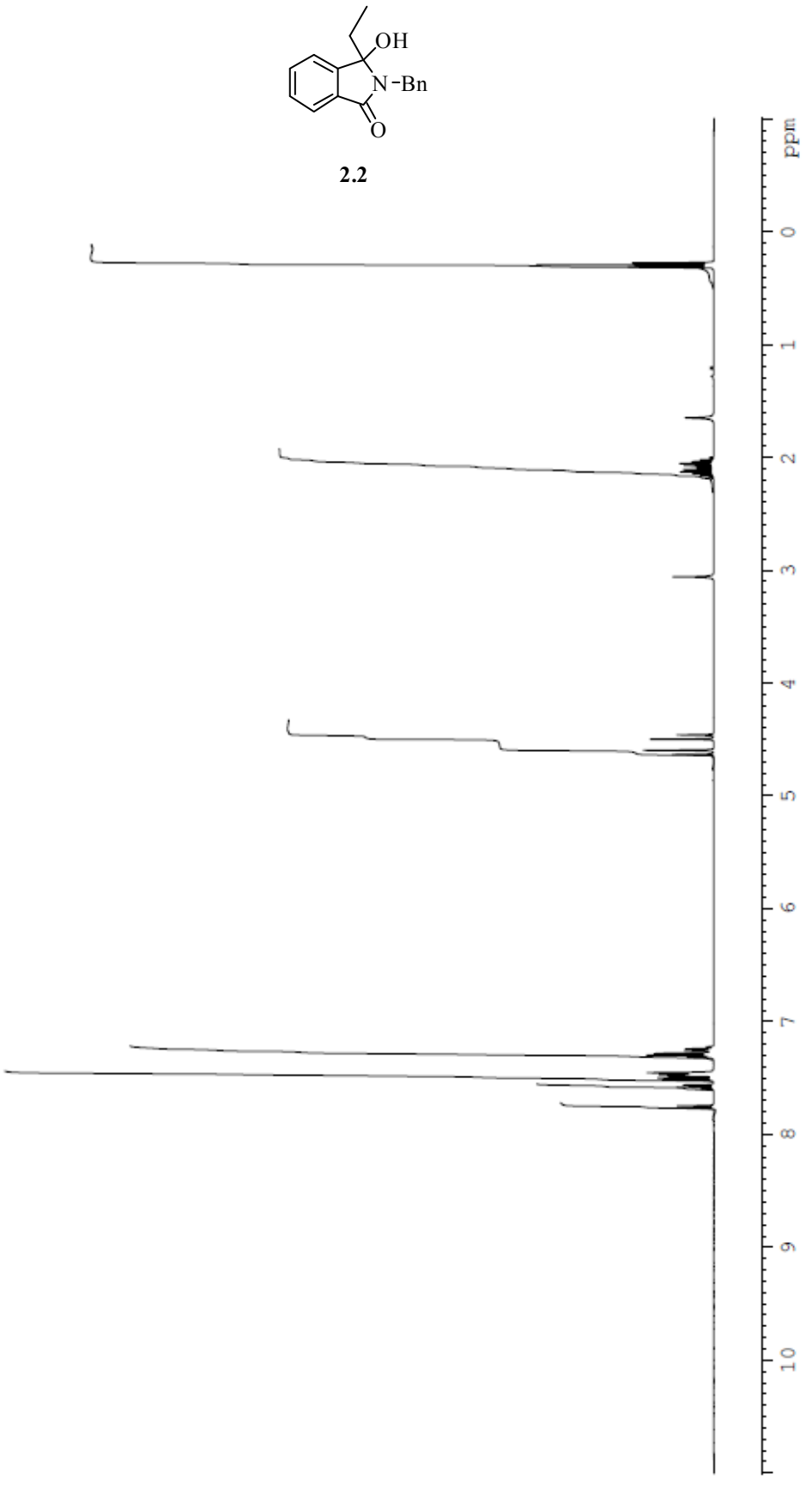
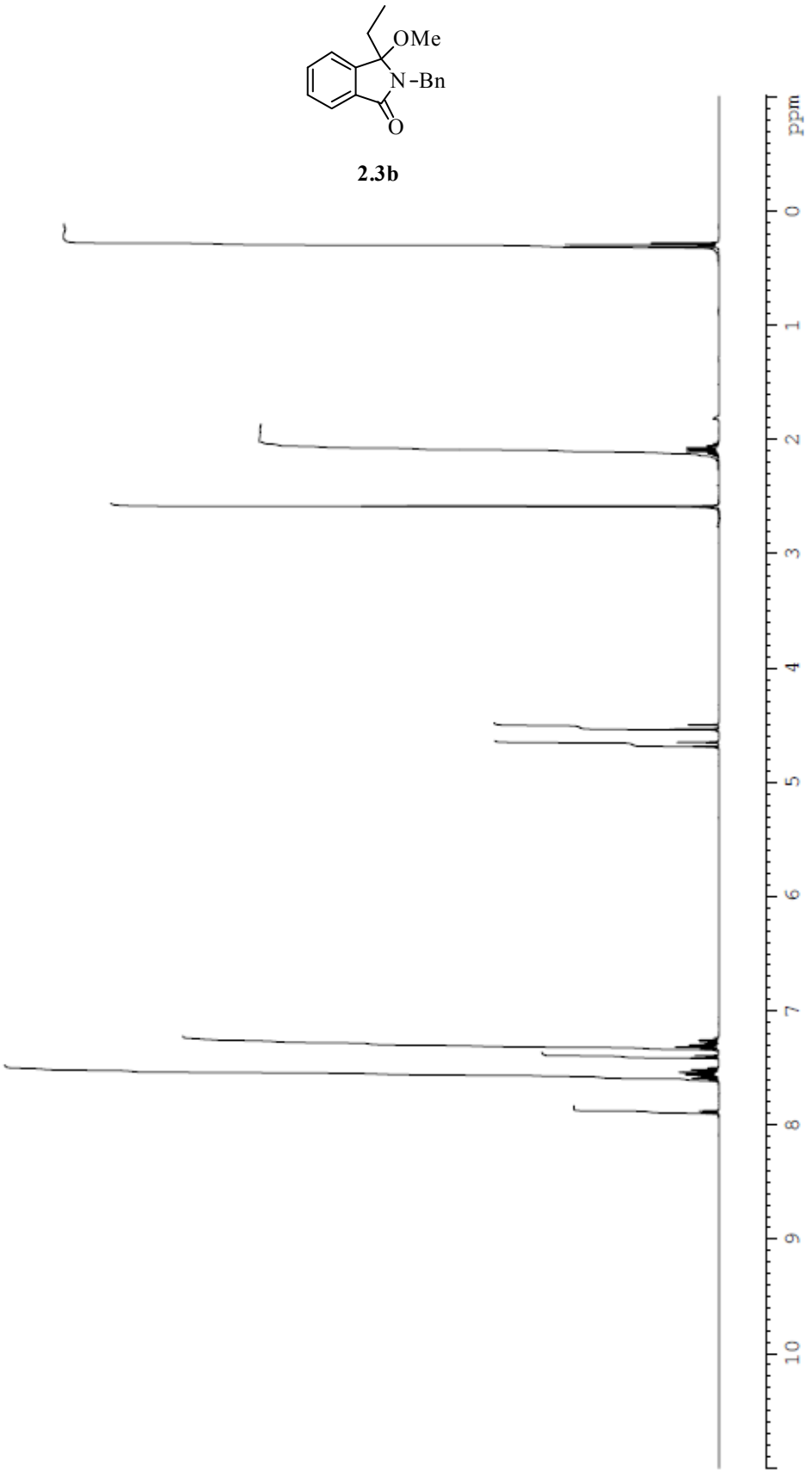


Figure A7: ^{1}H-NMR spectrum of **2.2** ($CDCl_3$)



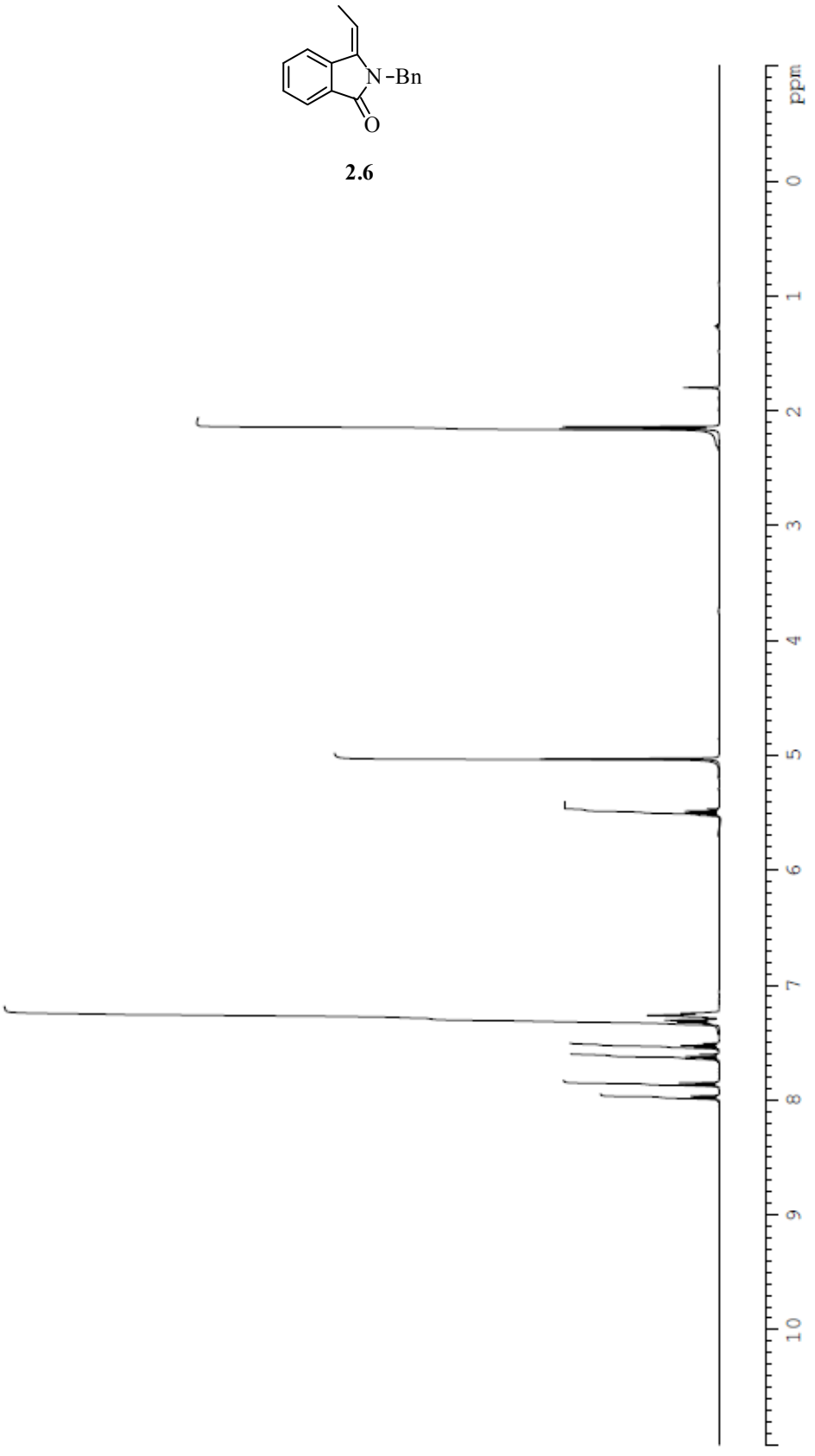
2.2

Figure A8: ^{1}H-NMR spectrum of **2.3b** ($CDCl_3$)



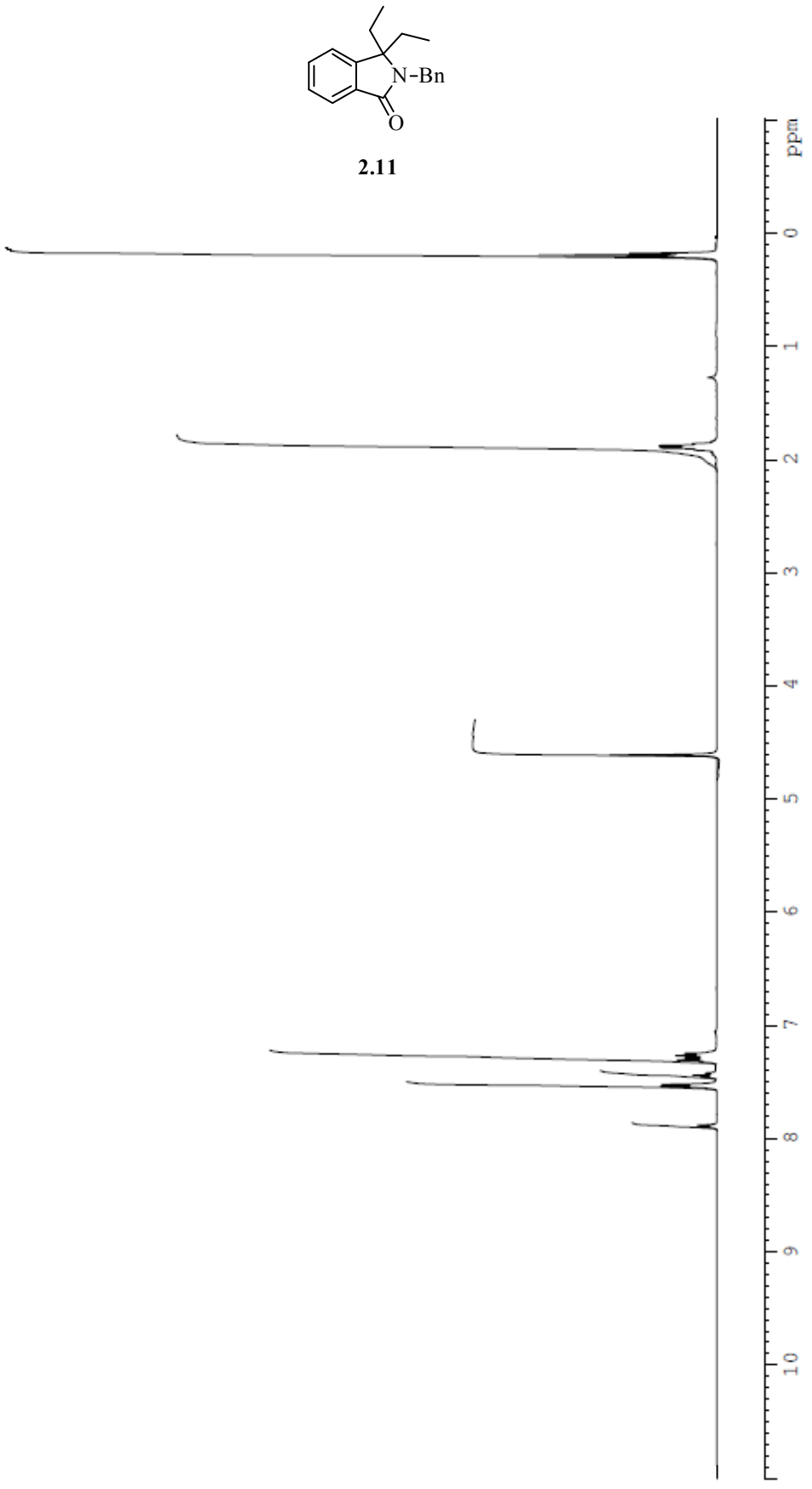
2.3b

Figure A9: ^{1}H-NMR spectrum of **2.6** ($CDCl_3$)



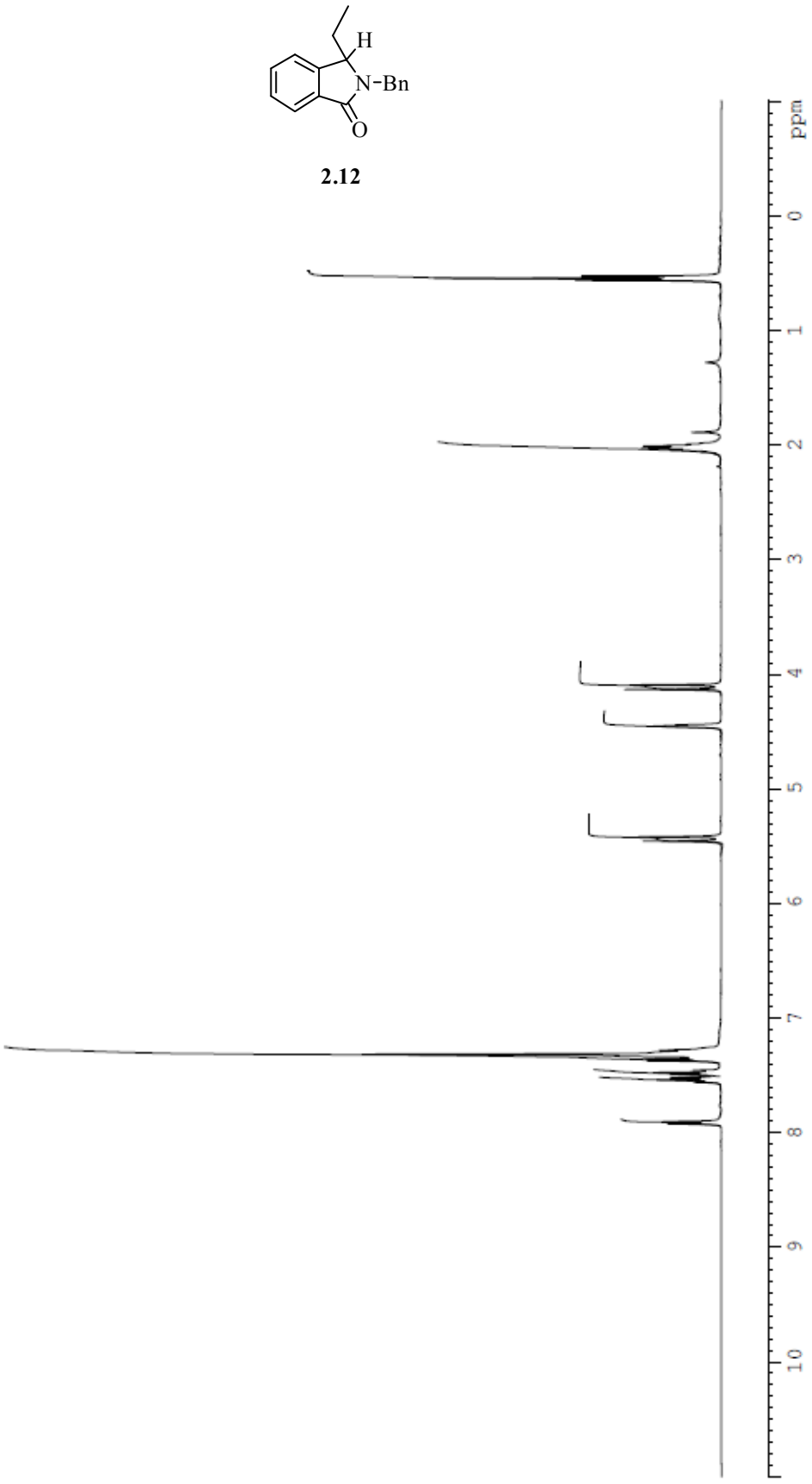
2.6

Figure A10: ^{1}H-NMR spectrum of **2.11** ($CDCl_3$)



2.11

Figure A11: ^{1}H-NMR spectrum of **2.12** ($CDCl_3$)



2.12

Figure A12: ^{1}H-NMR spectrum of **3.1** ($CDCl_3$)

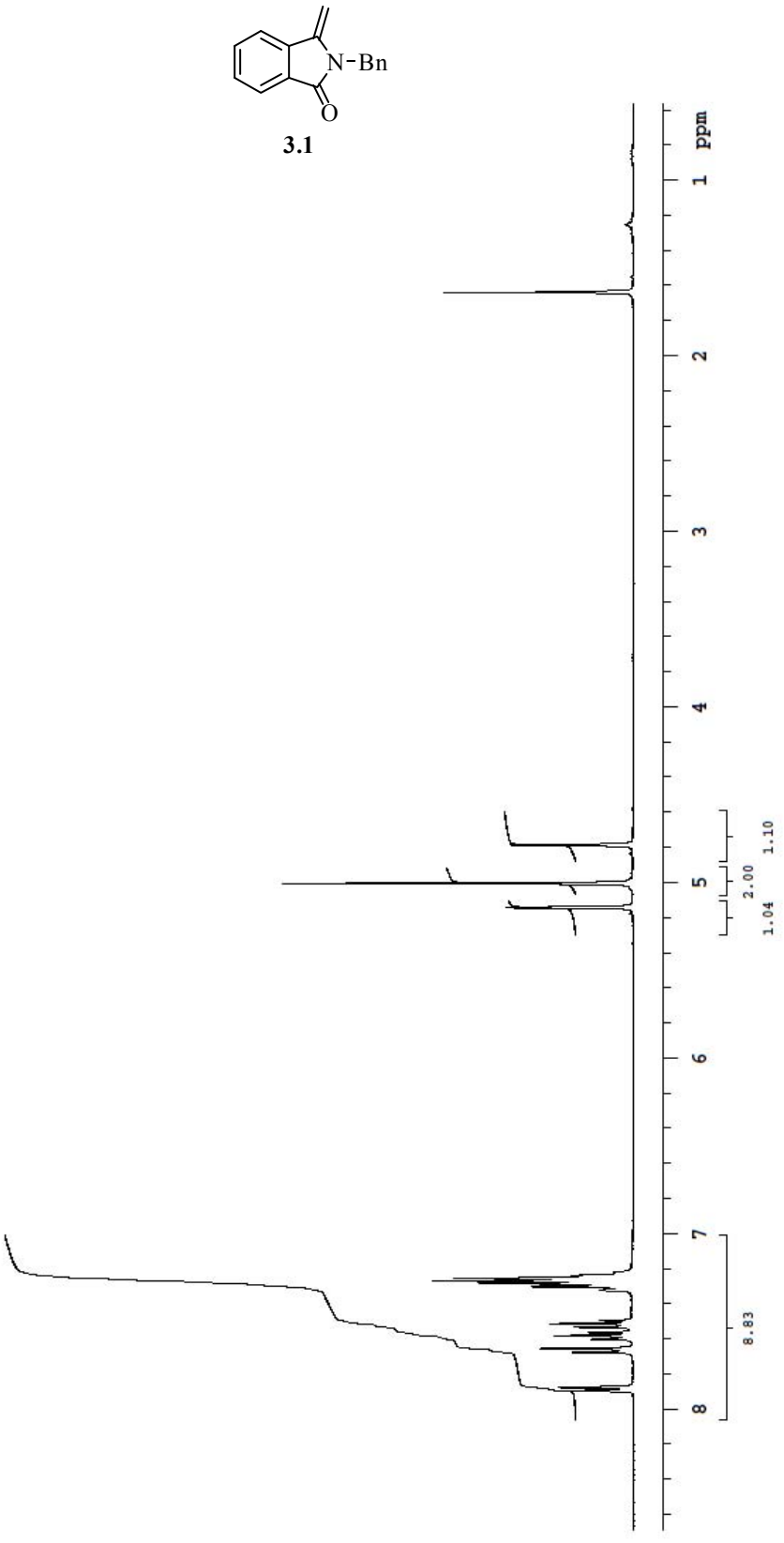


Figure A13: ^{1}H-NMR spectrum of **3.2** ($CDCl_3$)

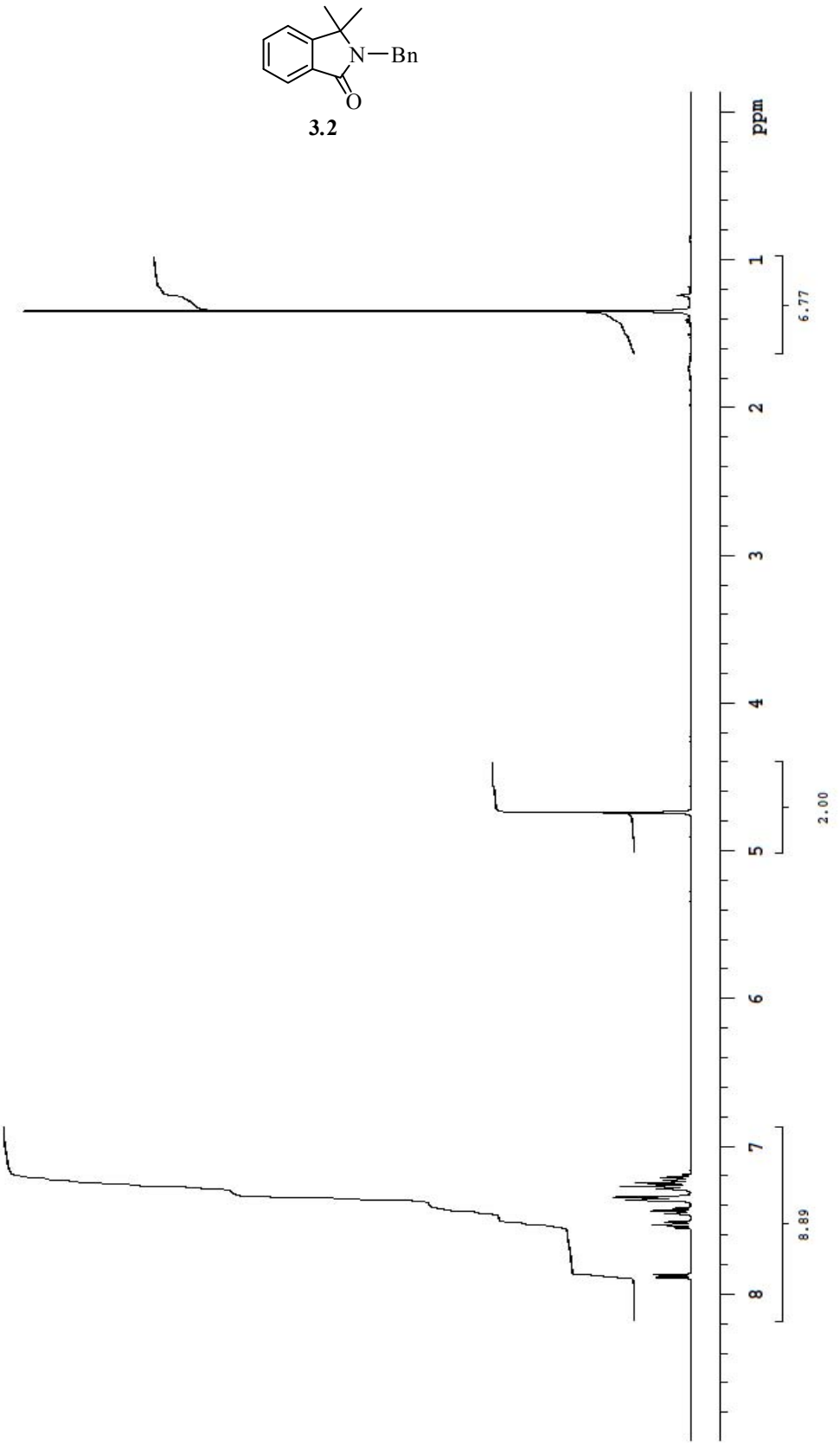
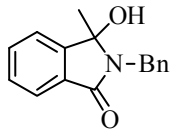


Figure A14: ^{1}H-NMR spectrum of **3.3** ($CDCl_3$)



3.3

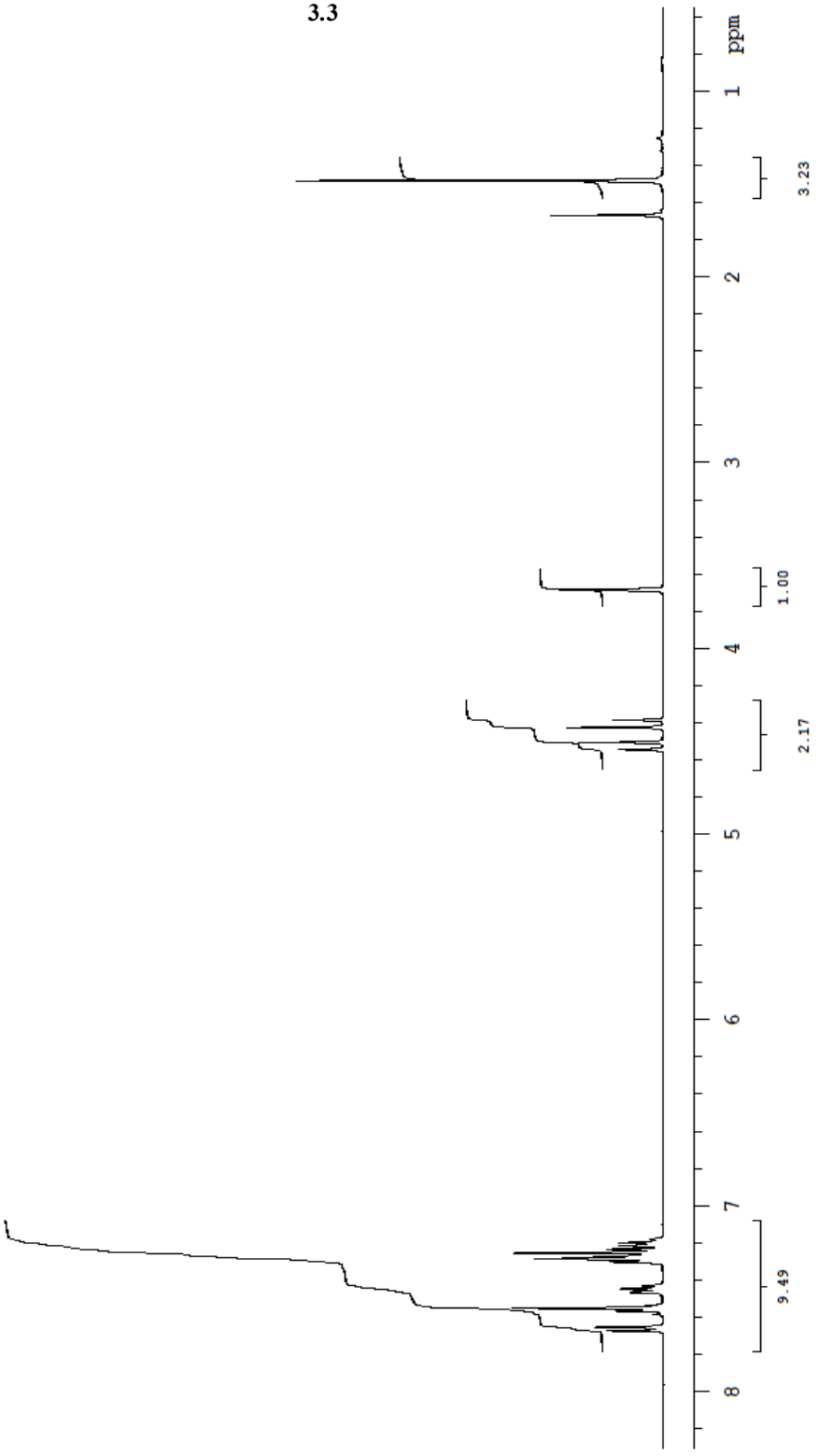


Figure A15: ^{1}H-NMR spectrum of **3.4b** ($CDCl_3$)

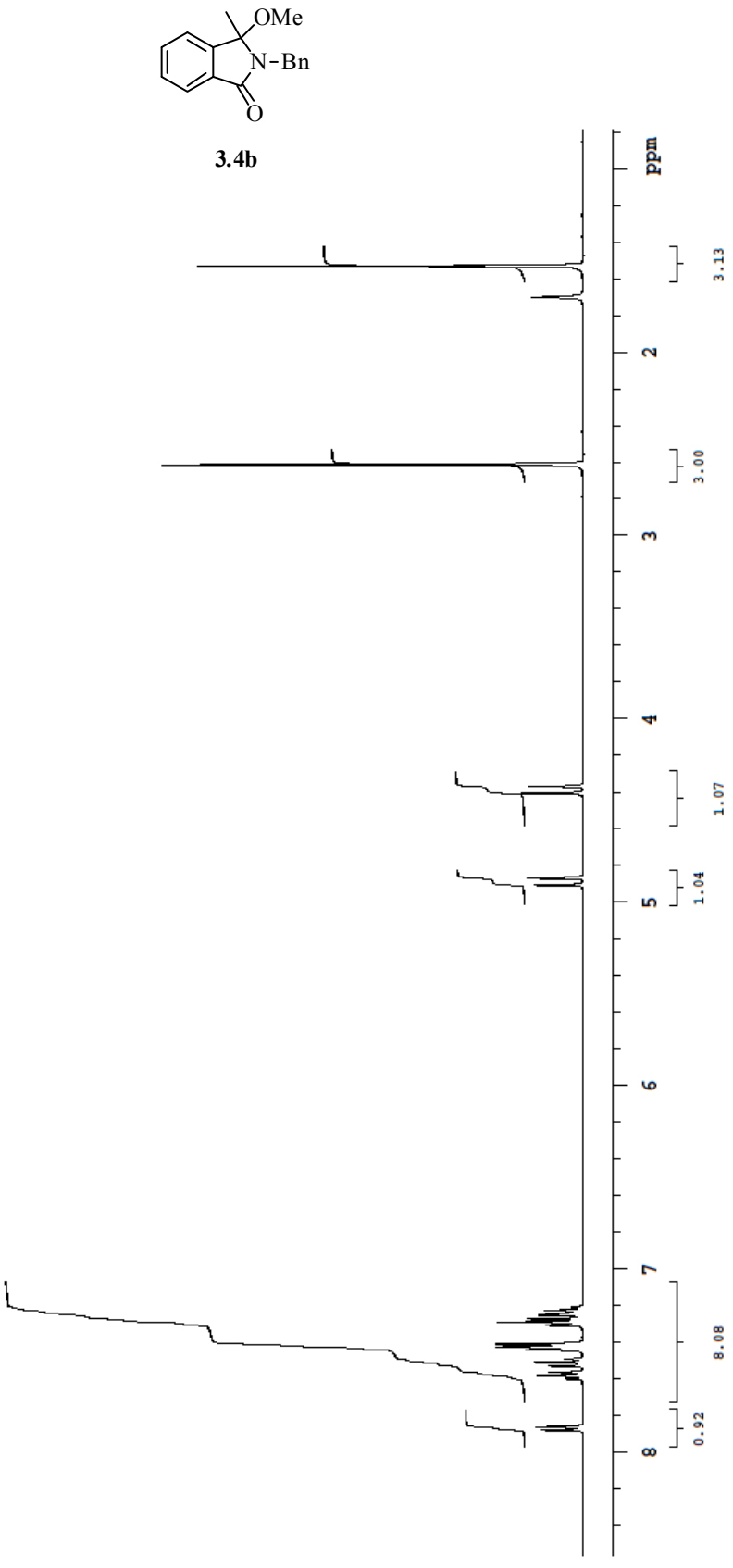


Figure A16: ^{1}H-NMR spectrum of **4.1b** ($CDCl_3$)

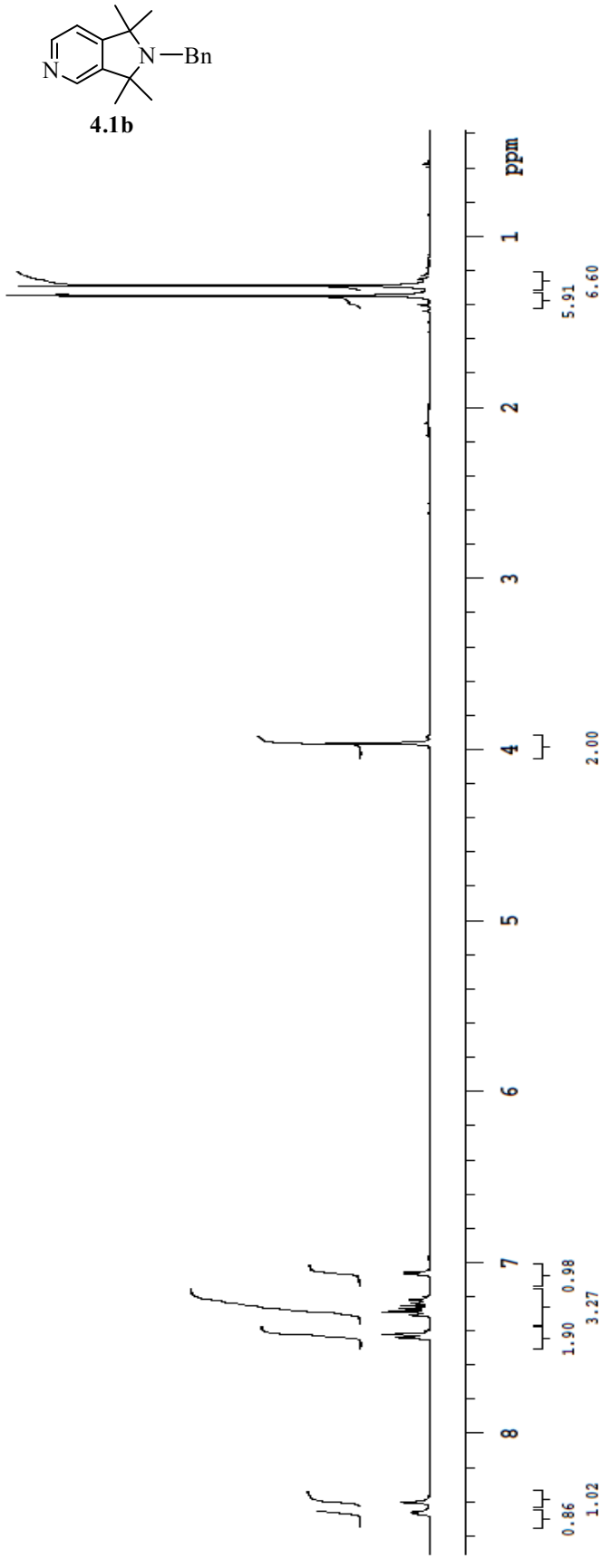


Figure A17: ^{1}H-NMR spectrum of **4.2** ($CDCl_3$)

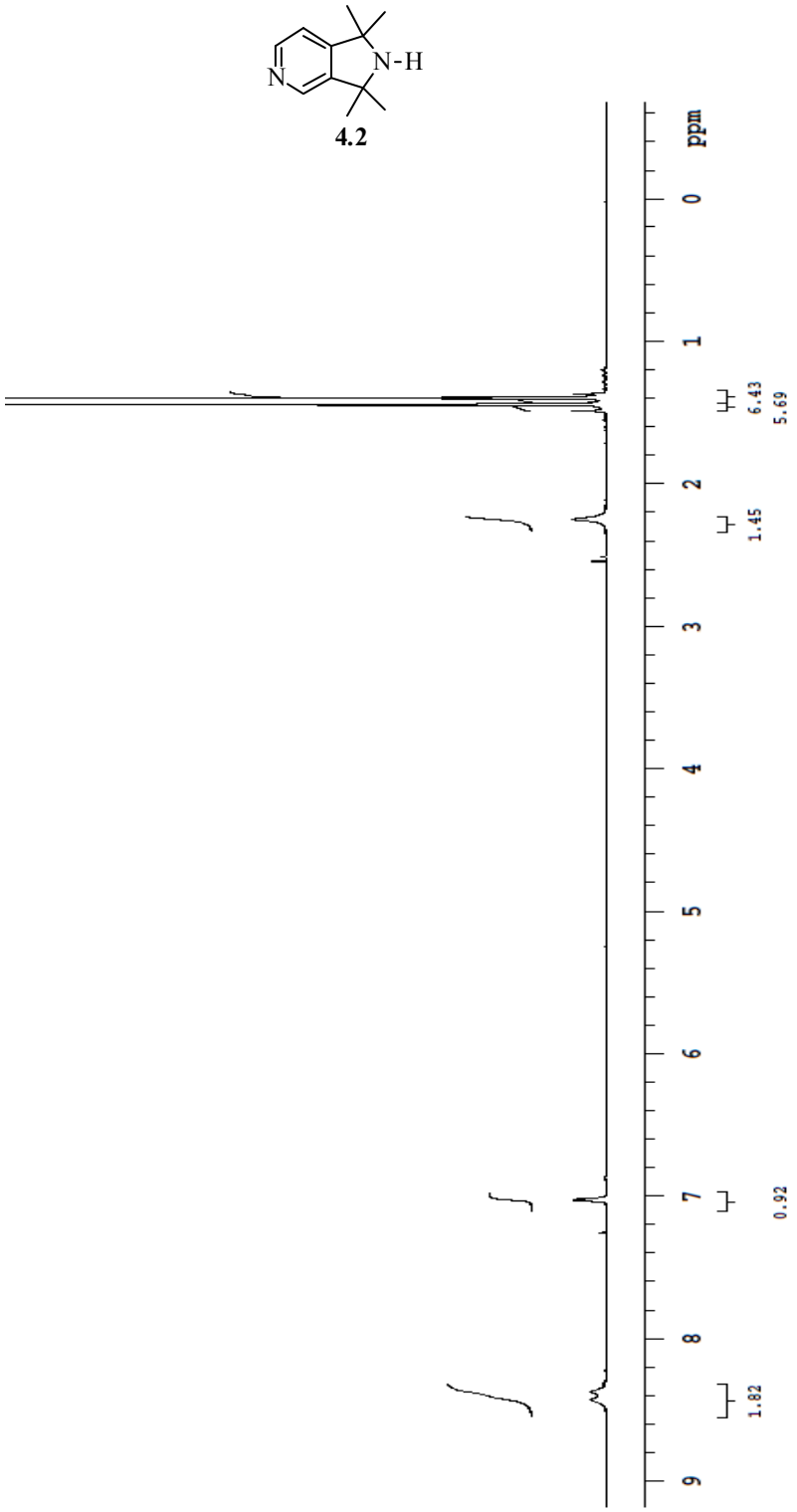


Figure A18: ^{1}H-NMR spectrum of **4.4** ($CDCl_3$)

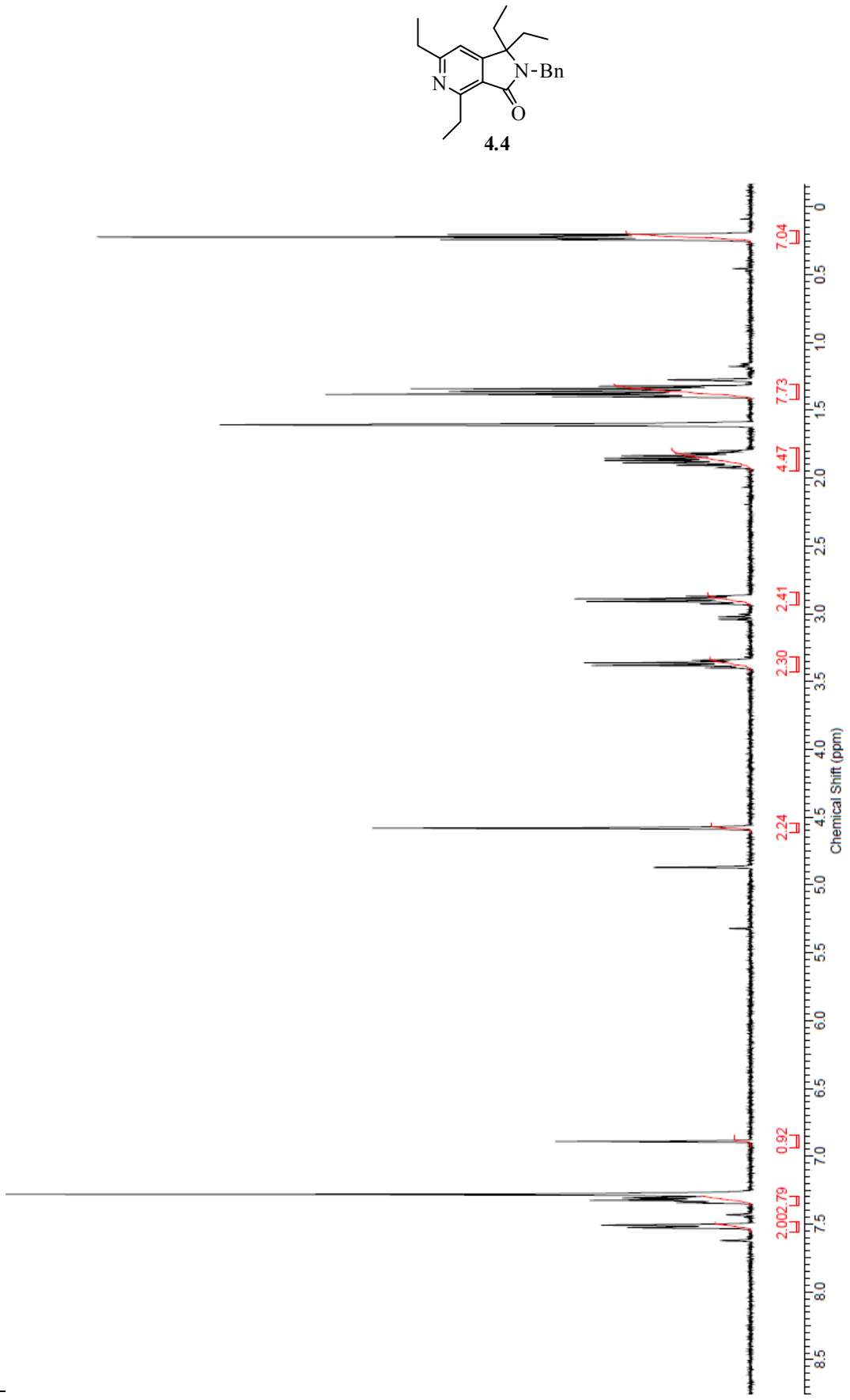
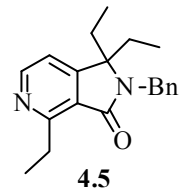


Figure A19: ^{1}H-NMR spectrum of **4.5** ($CDCl_3$)



4.5

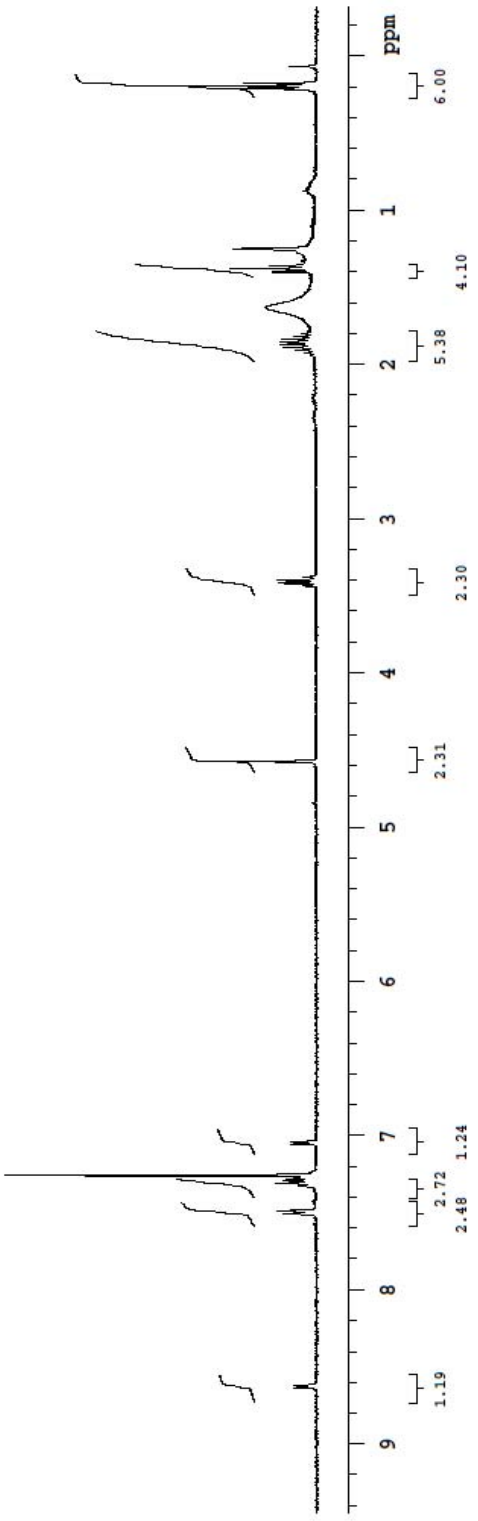


Figure A20: ^{1}H-NMR spectrum of **4.6** ($CDCl_3$)

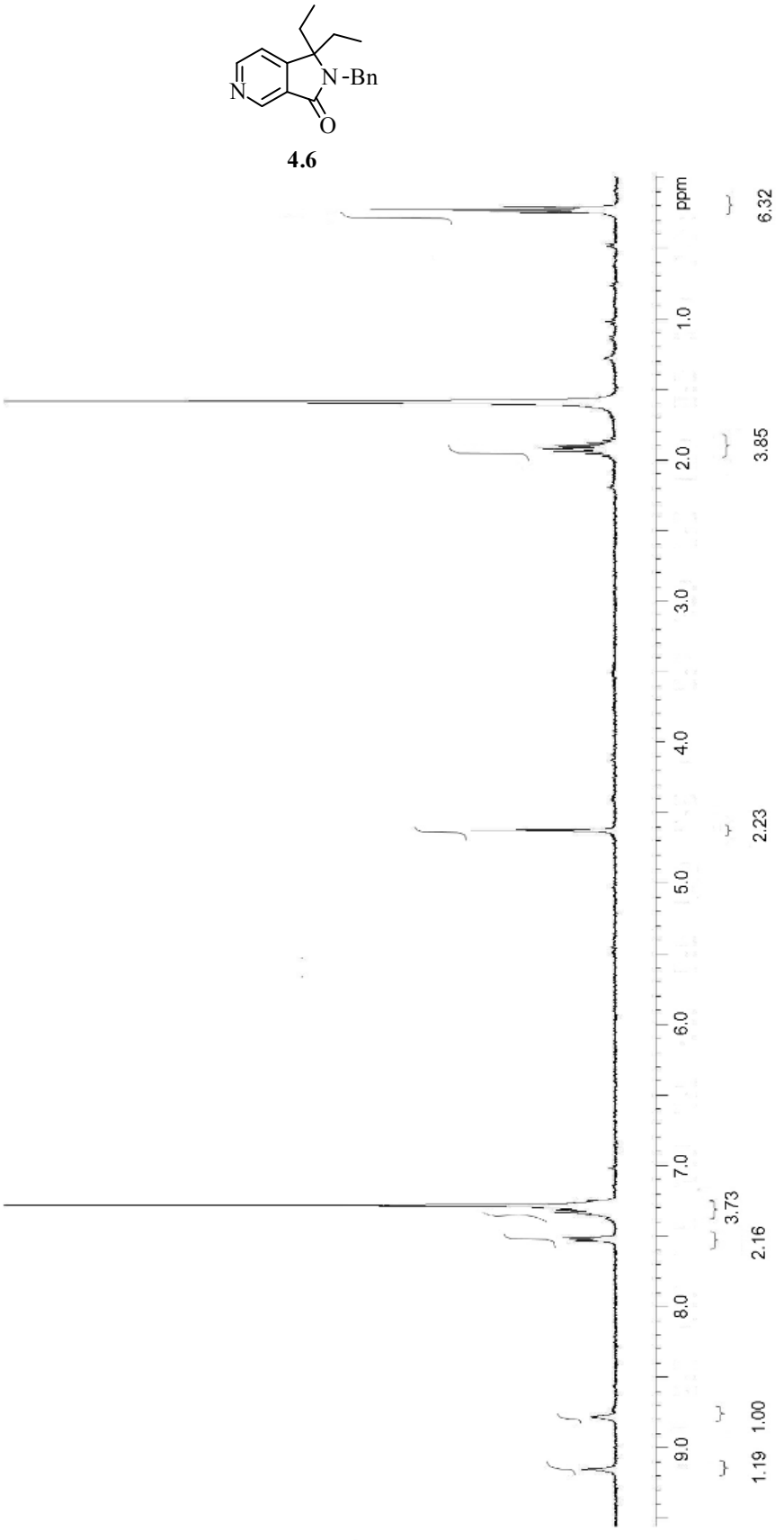


Figure A21: ^{1}H-NMR spectrum of **4.7** ($CDCl_3$)

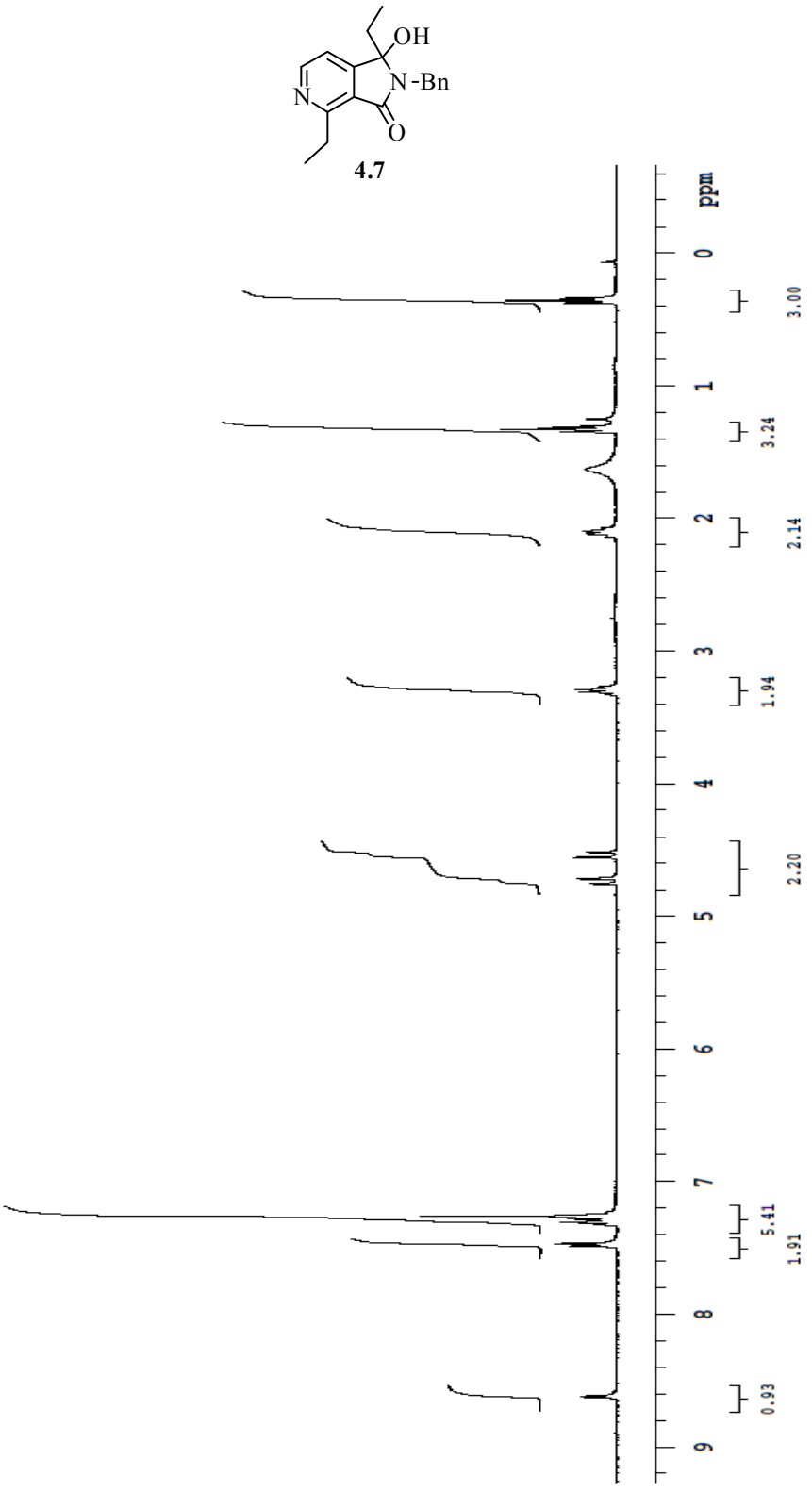


Figure A22: ^{1}H-NMR spectrum of **4.8** ($CDCl_3$)

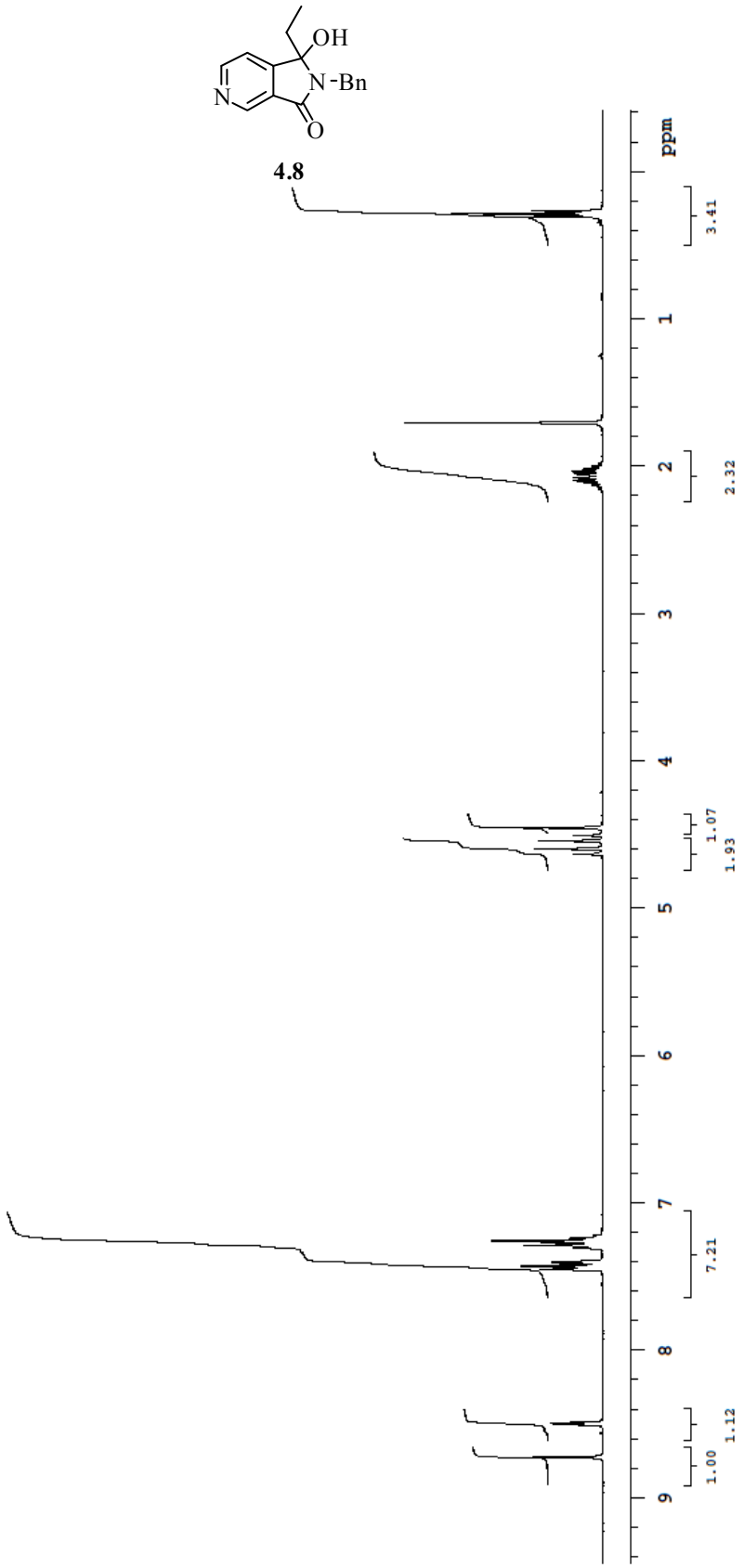


Figure A23: ^{1}H-NMR spectrum of **4.14** ($CDCl_3$)

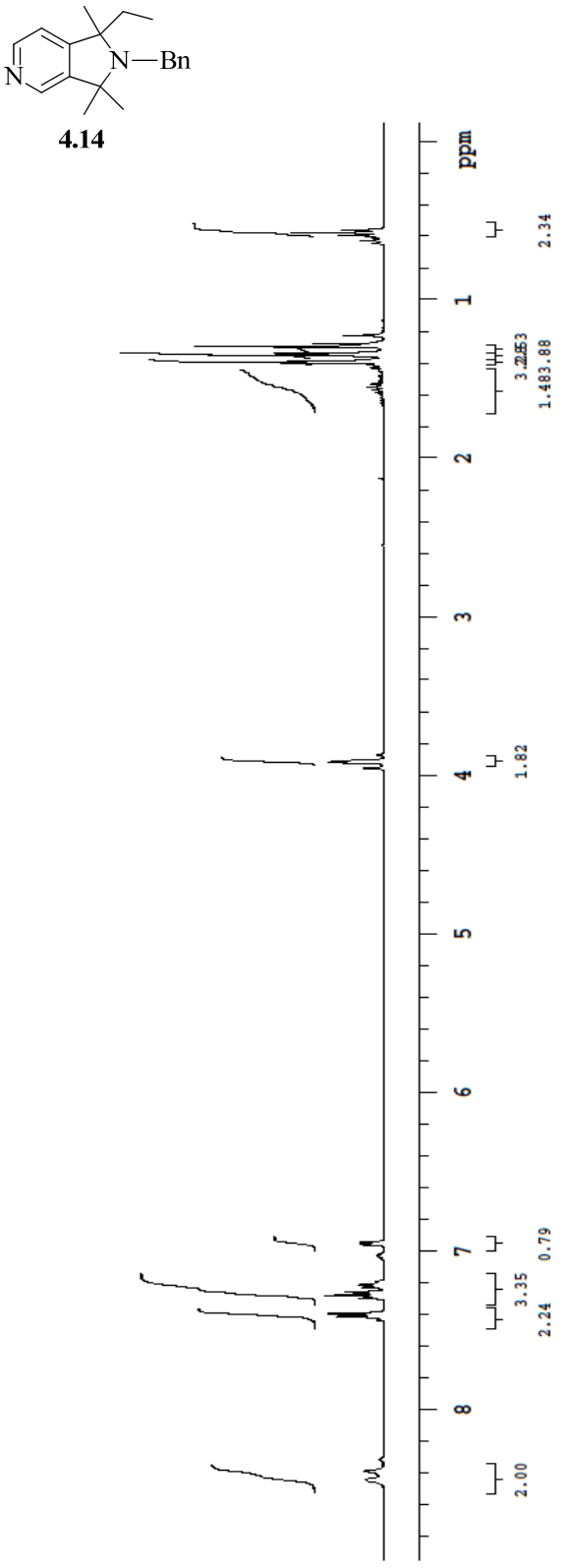
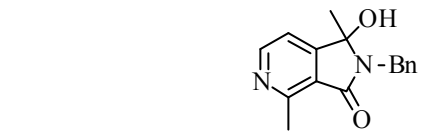


Figure A24: ^{1}H-NMR spectrum of **4.15** ($CDCl_3$)



4.15

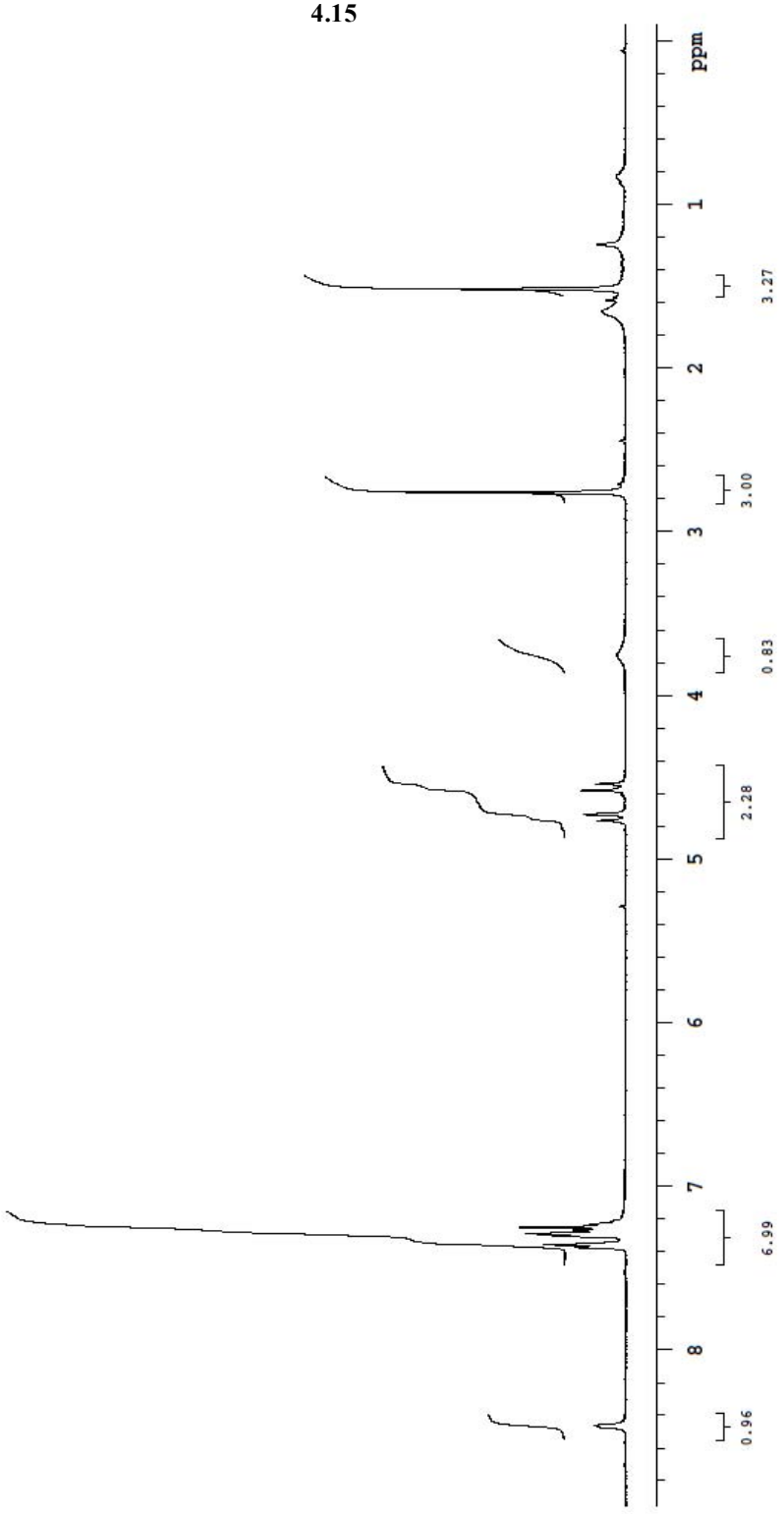
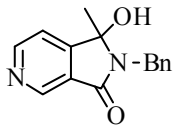


Figure A25: ^{1}H-NMR spectrum of **4.16** ($CDCl_3$)



4.16

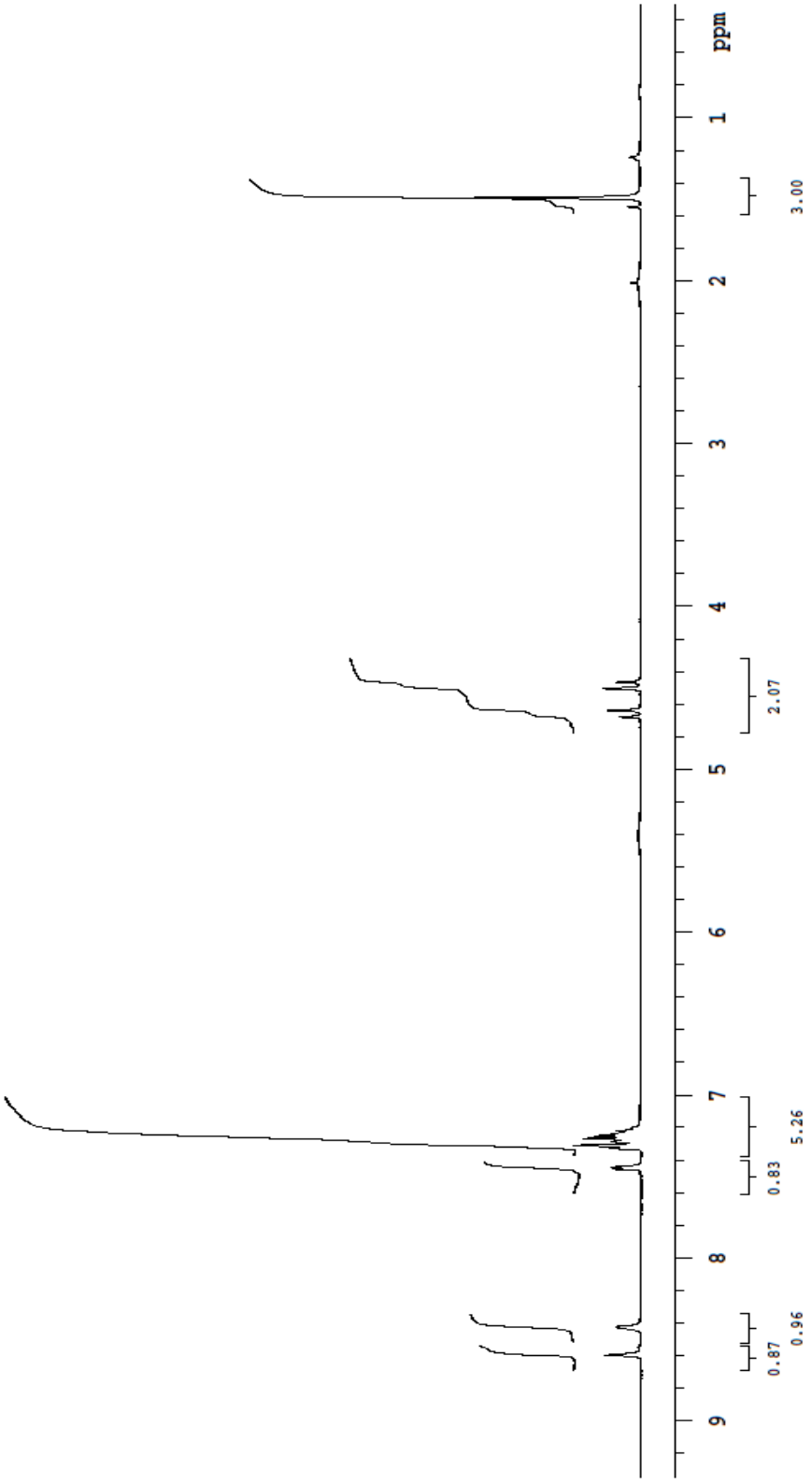
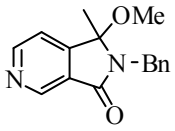
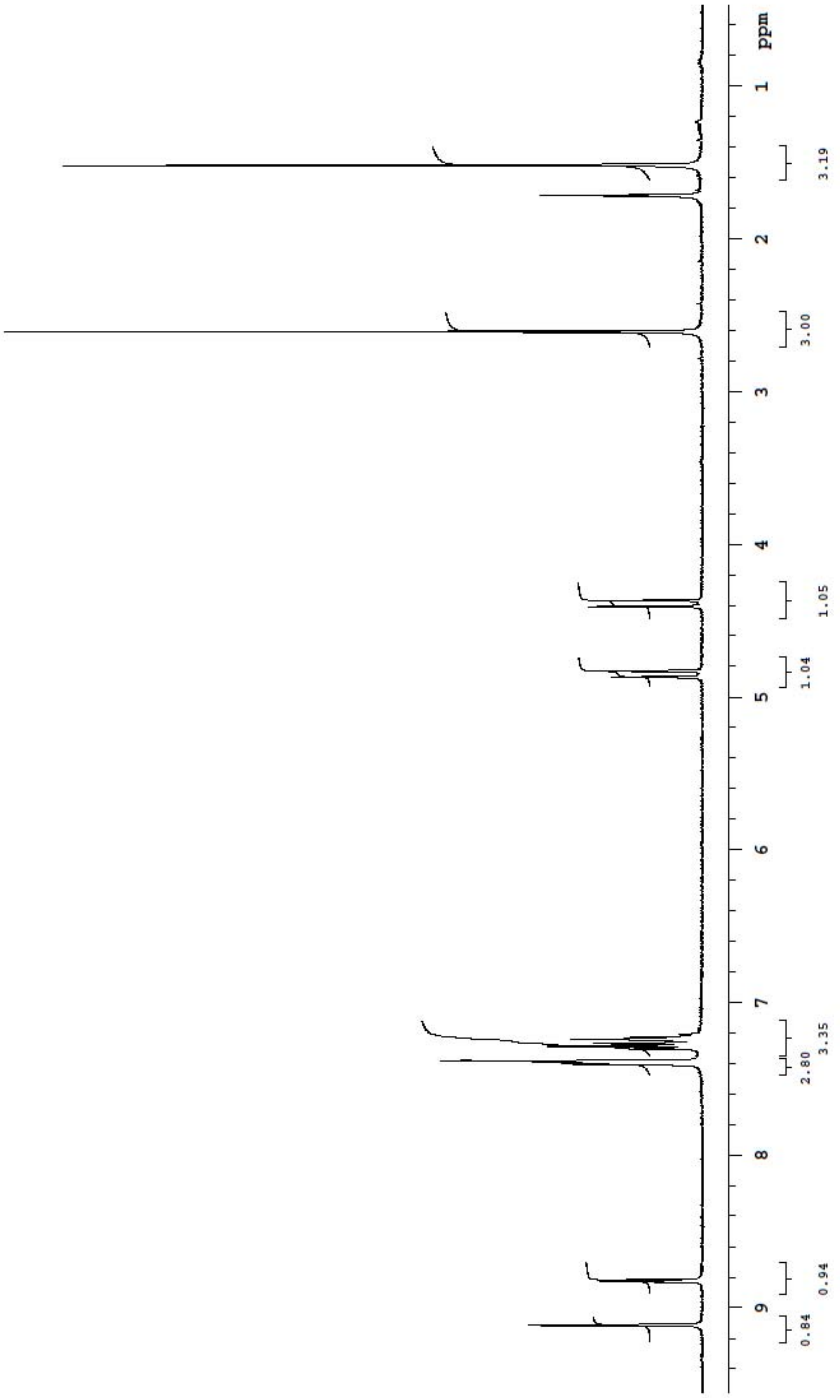


Figure A26: ^{1}H-NMR spectrum of **4.17b** ($CDCl_3$)



4.17b



8.2 ^{13}C-NMR SPECTRA

Figure A27: ^{13}C-NMR spectrum of **1.75** ($CDCl_3$)

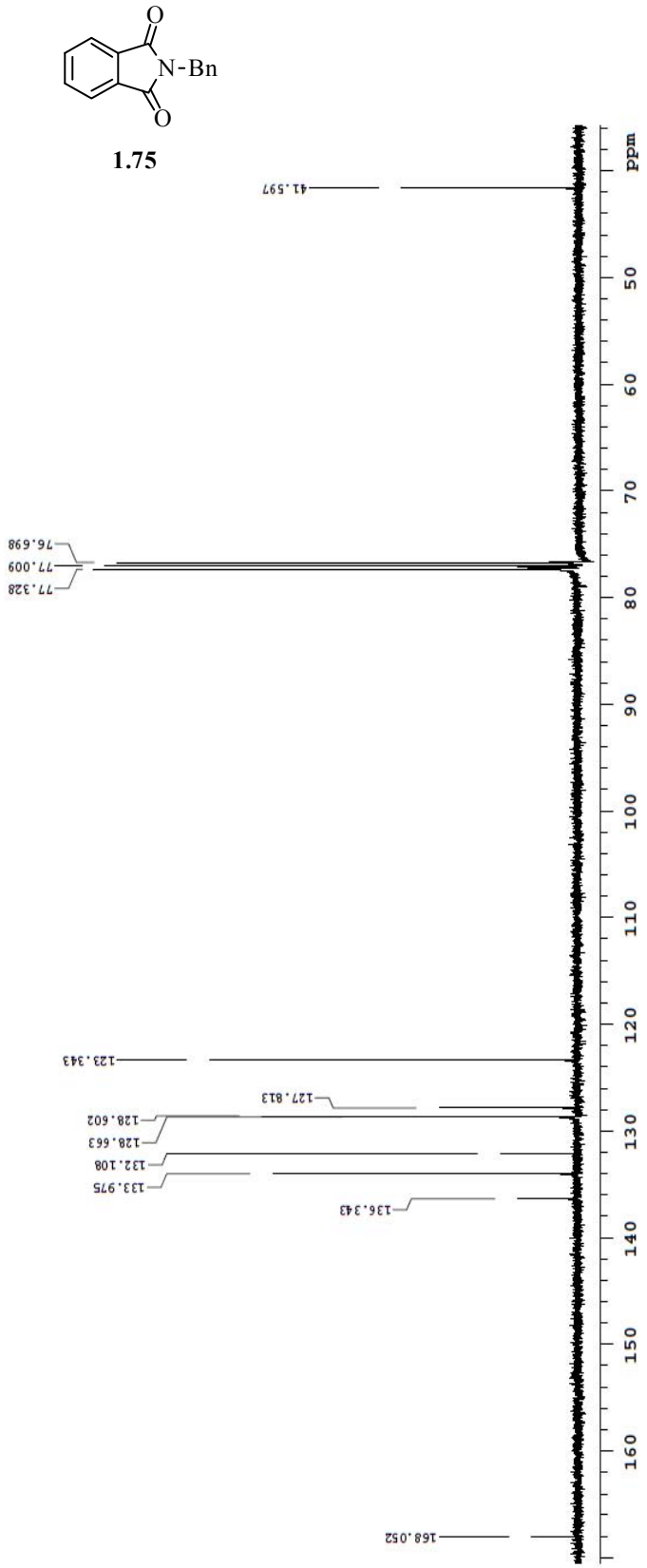


Figure A28: ^{13}C-NMR spectrum of **1.76** ($CDCl_3$)

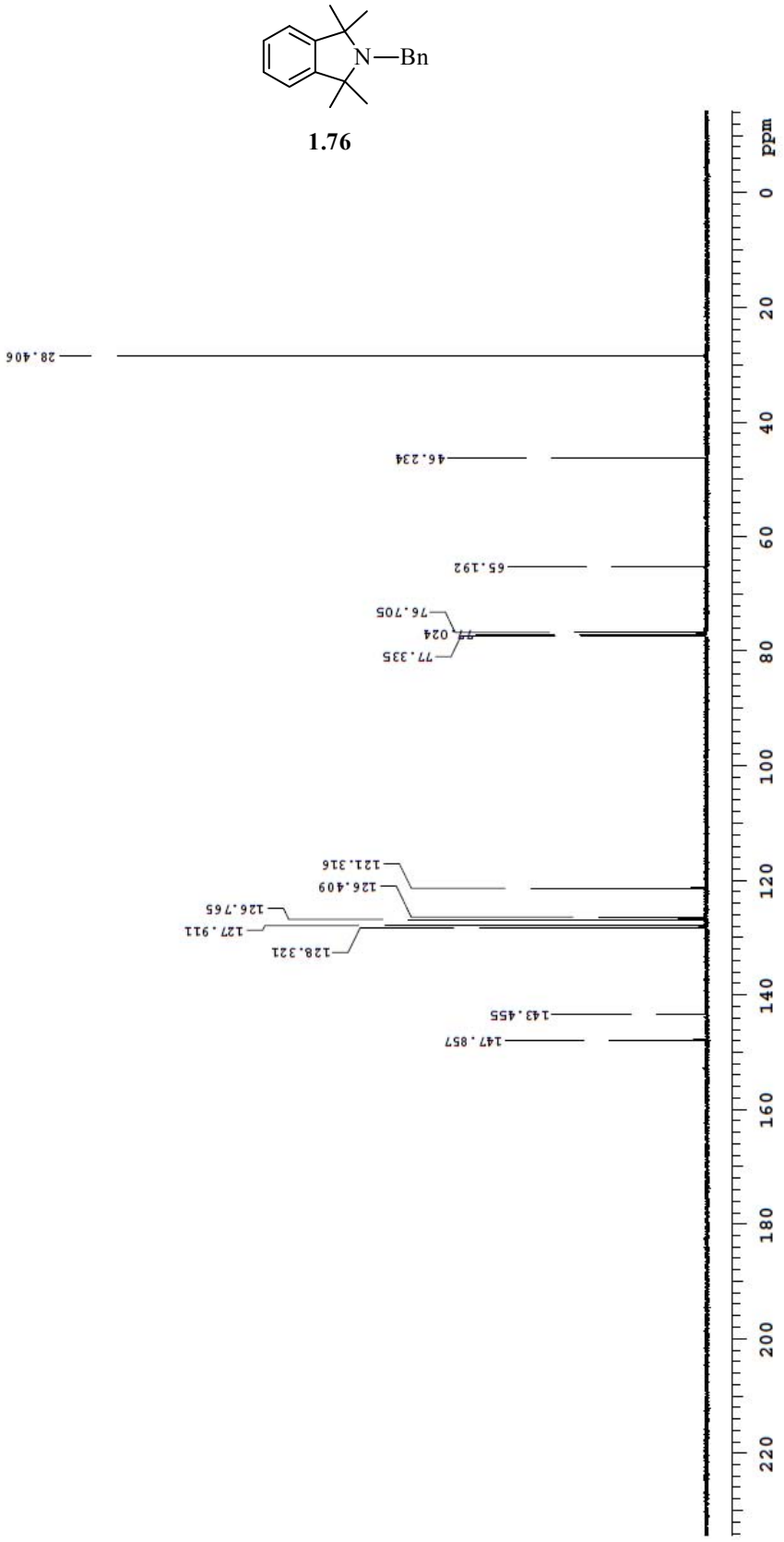
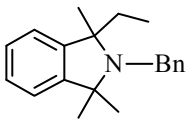


Figure A29: ^{13}C-NMR spectrum of **1.82** ($CDCl_3$)



1.82

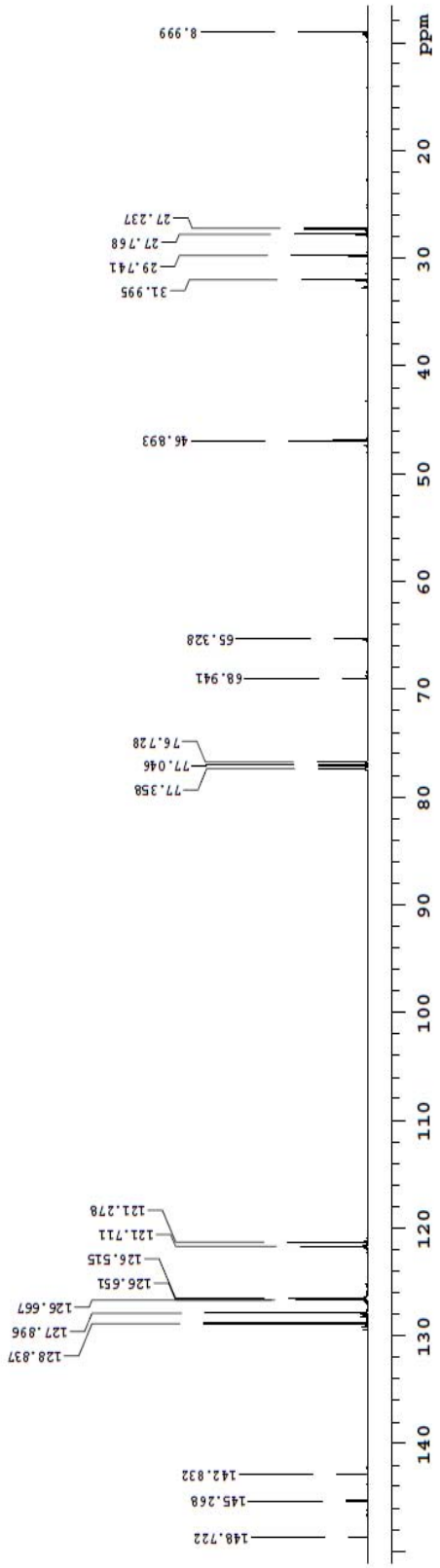


Figure A30: ^{13}C-NMR spectrum of **1.94** ($CDCl_3$)

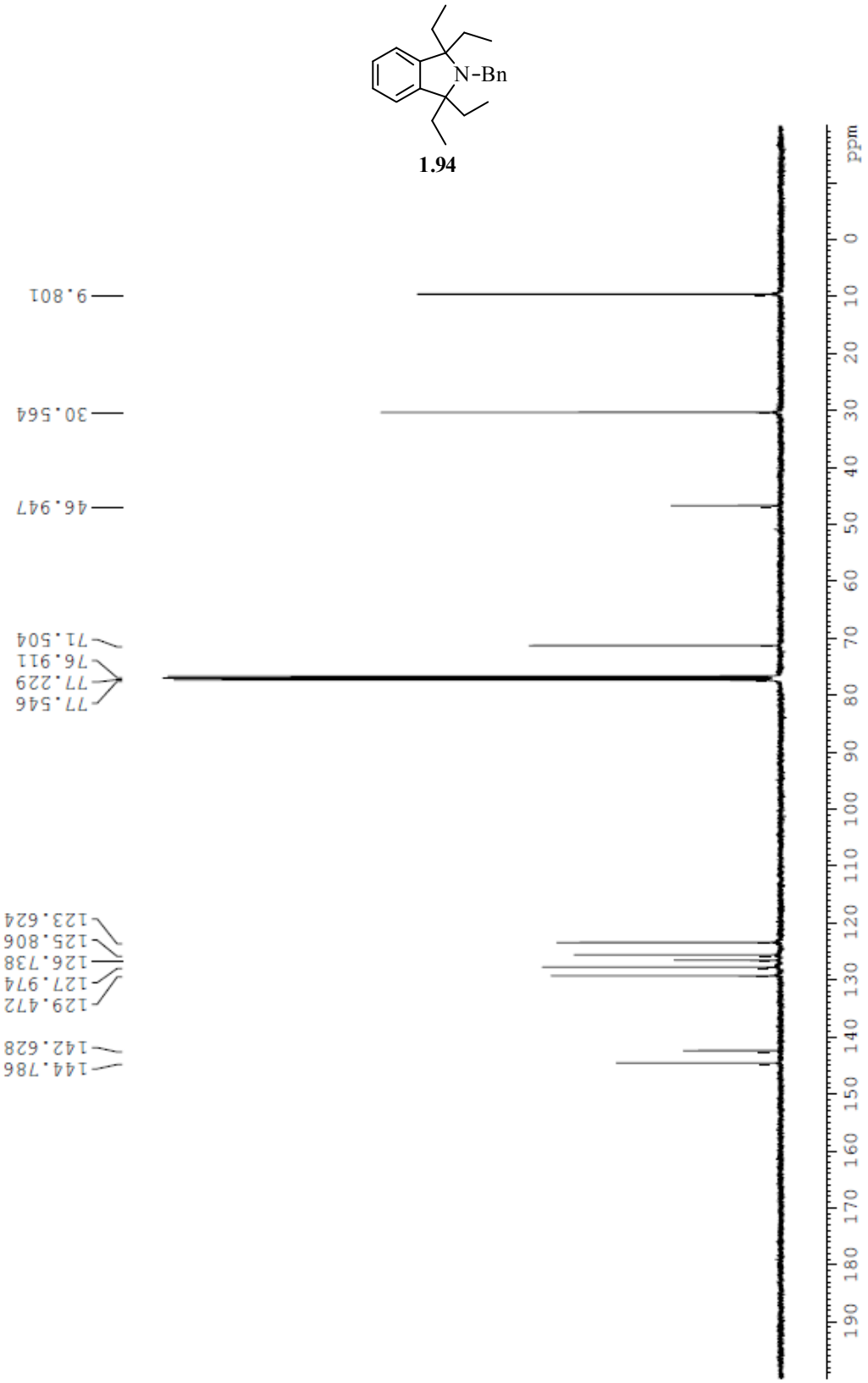
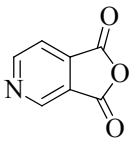


Figure A31: ^{13}C-NMR spectrum of **1.108** ($CDCl_3$)



1.108

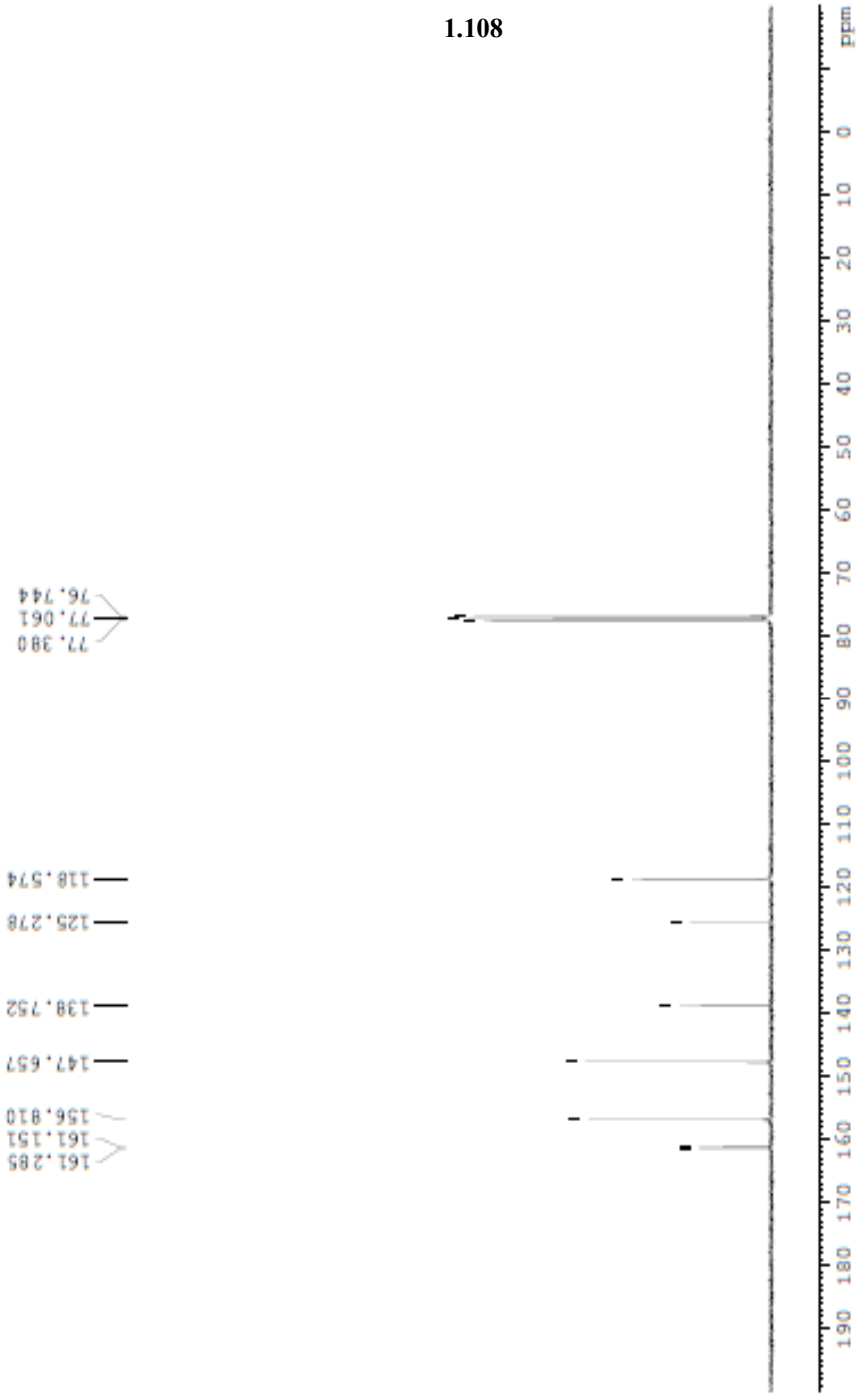


Figure A32: ^{13}C-NMR spectrum of **1.109** ($CDCl_3$)

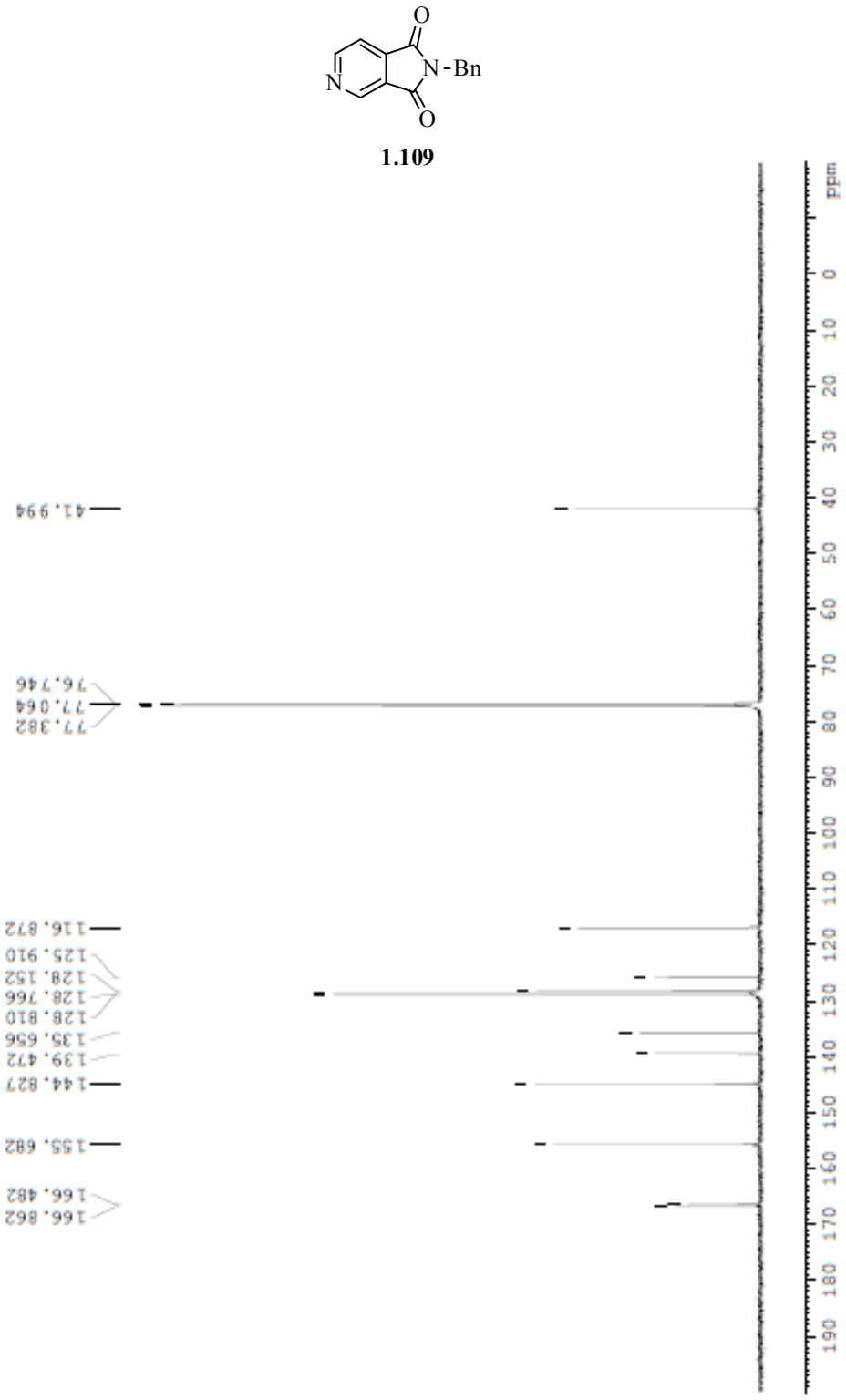


Figure A33: ^{13}C-NMR spectrum of **2.2** ($CDCl_3$)

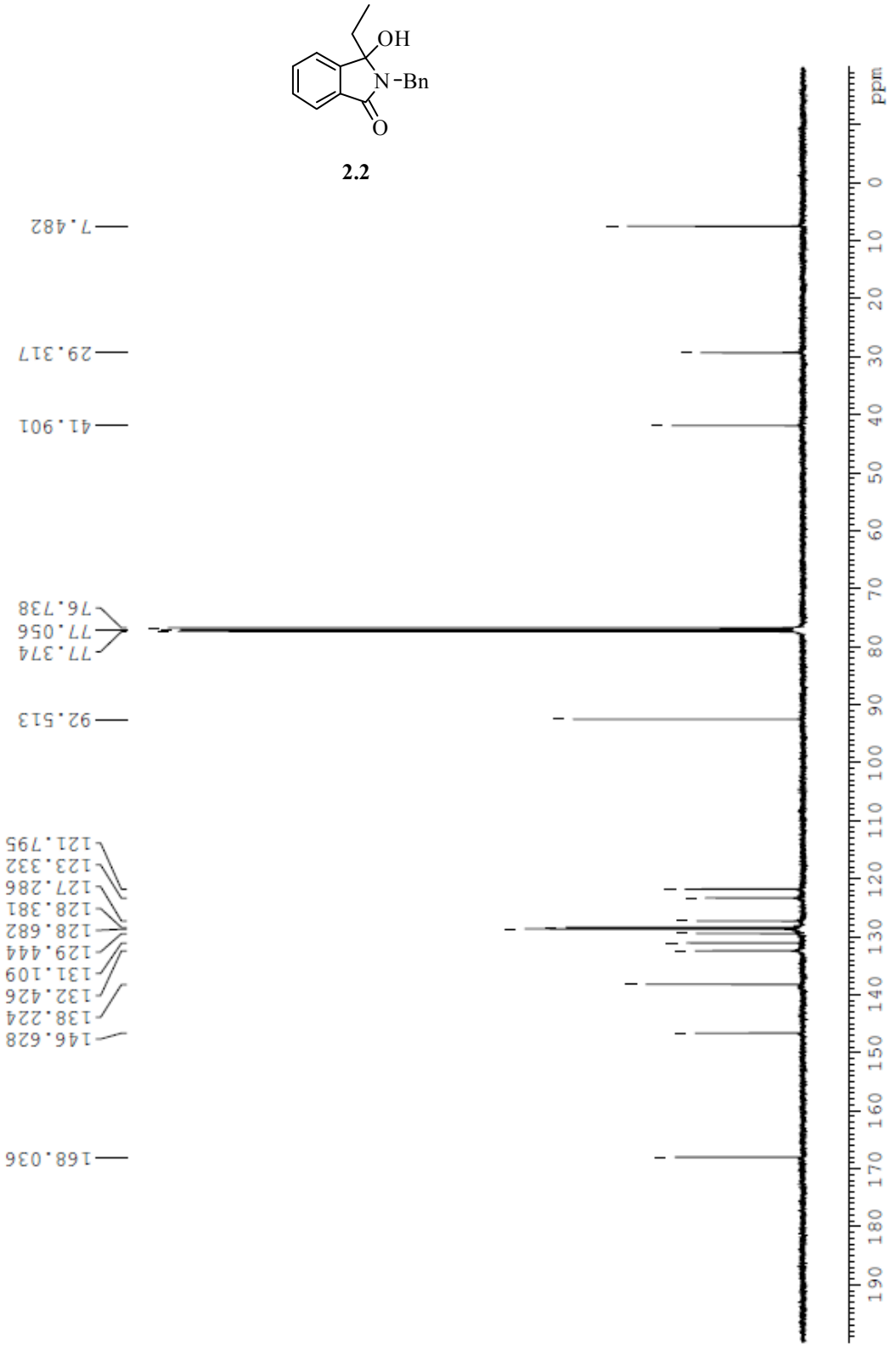


Figure A34: ^{13}C-NMR spectrum of **2.3b** ($CDCl_3$)

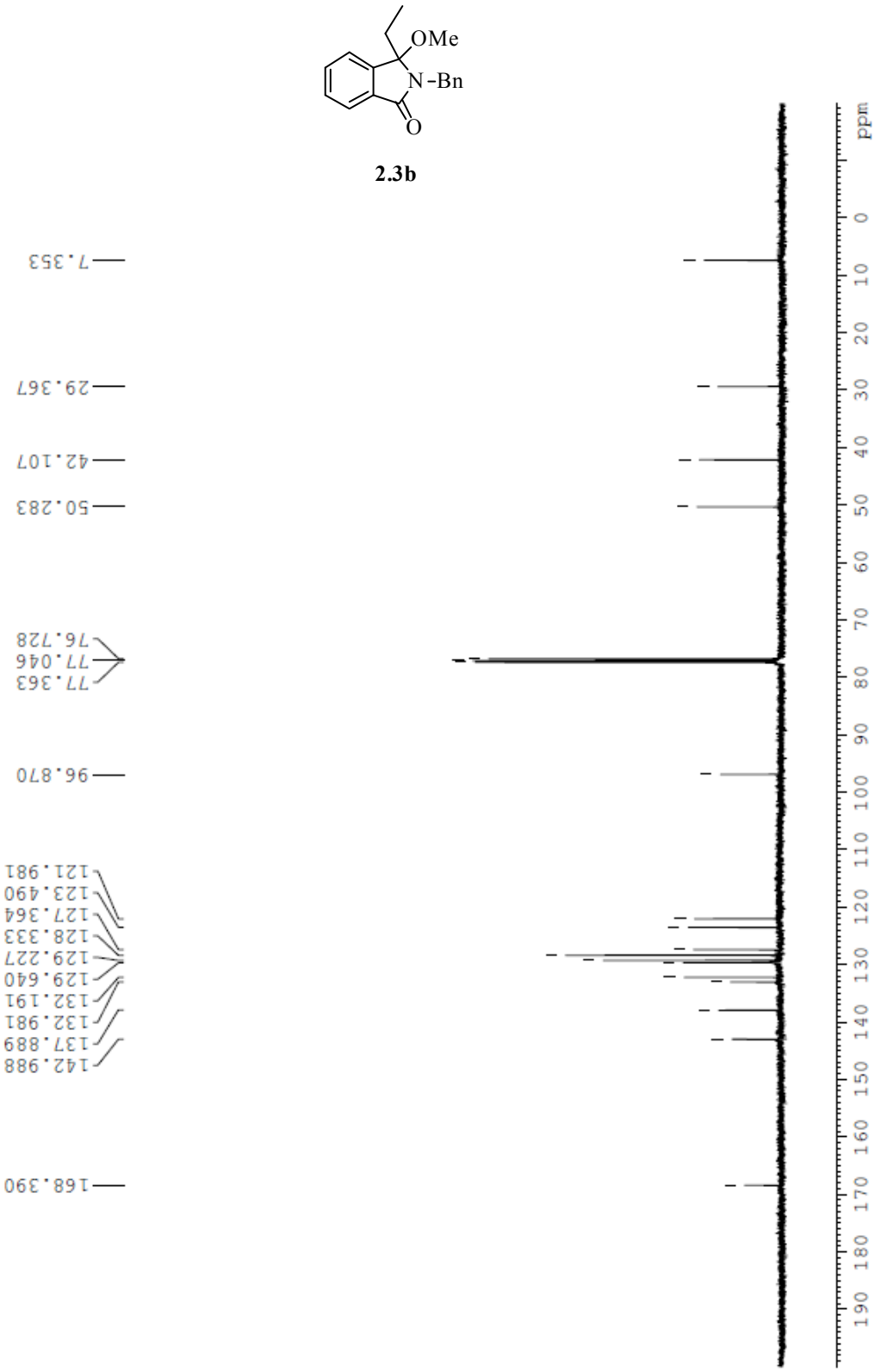


Figure A35: ^{13}C-NMR spectrum of **2.6** ($CDCl_3$)

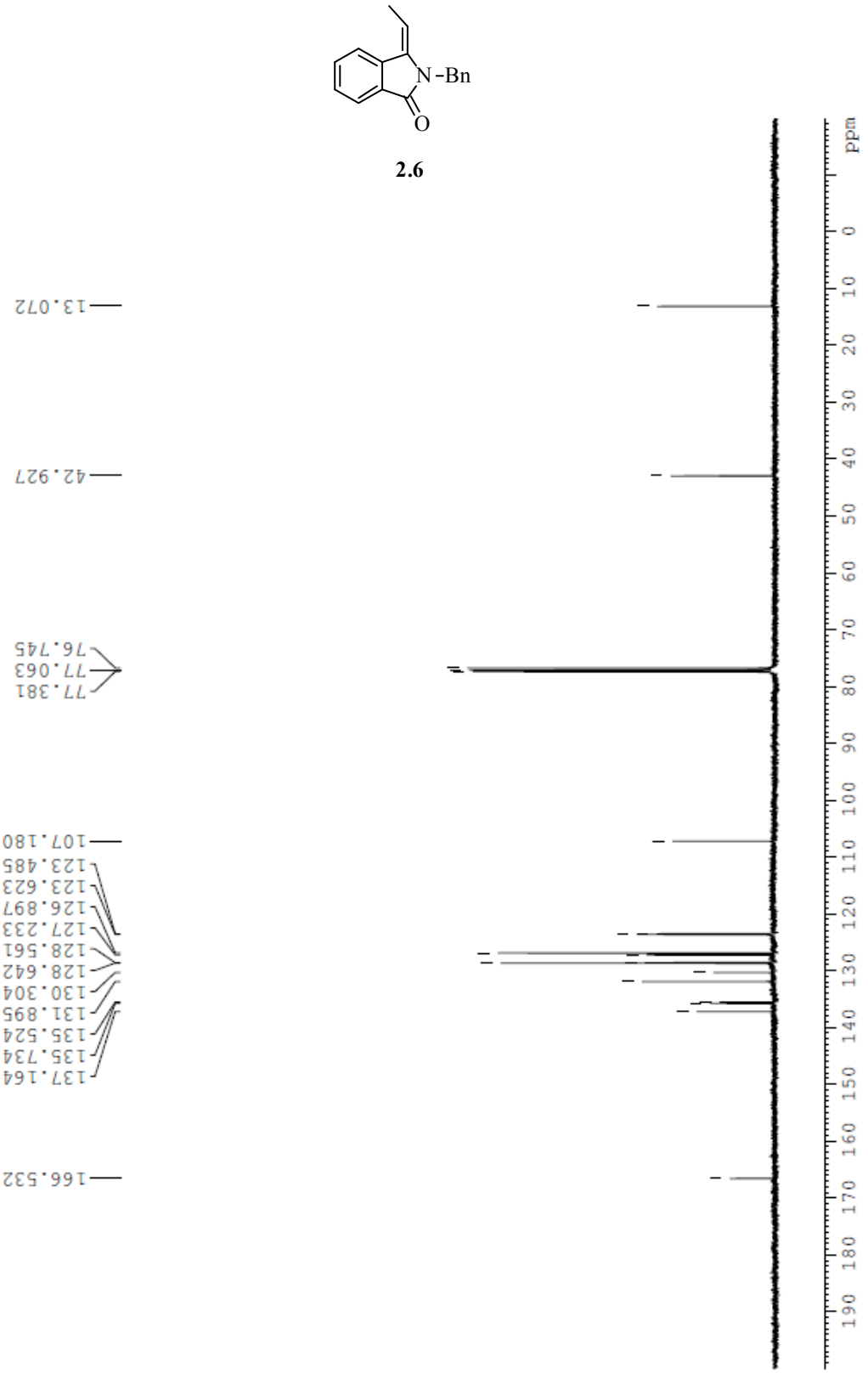
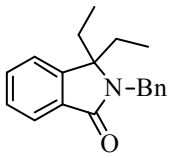


Figure A36: ^{13}C-NMR spectrum of **2.11** ($CDCl_3$)



2.11

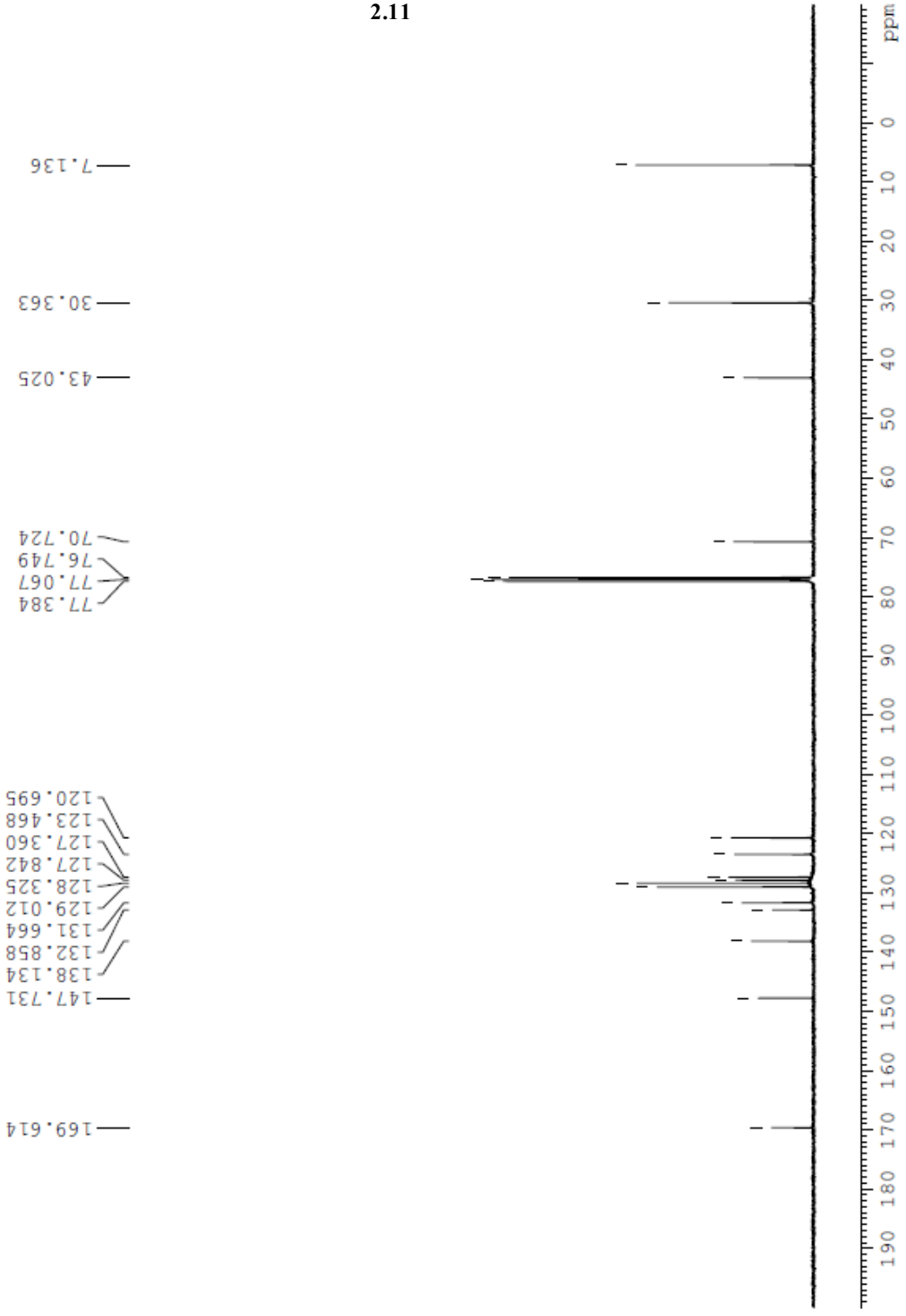


Figure A37: ^{13}C-NMR spectrum of **2.12** ($CDCl_3$)

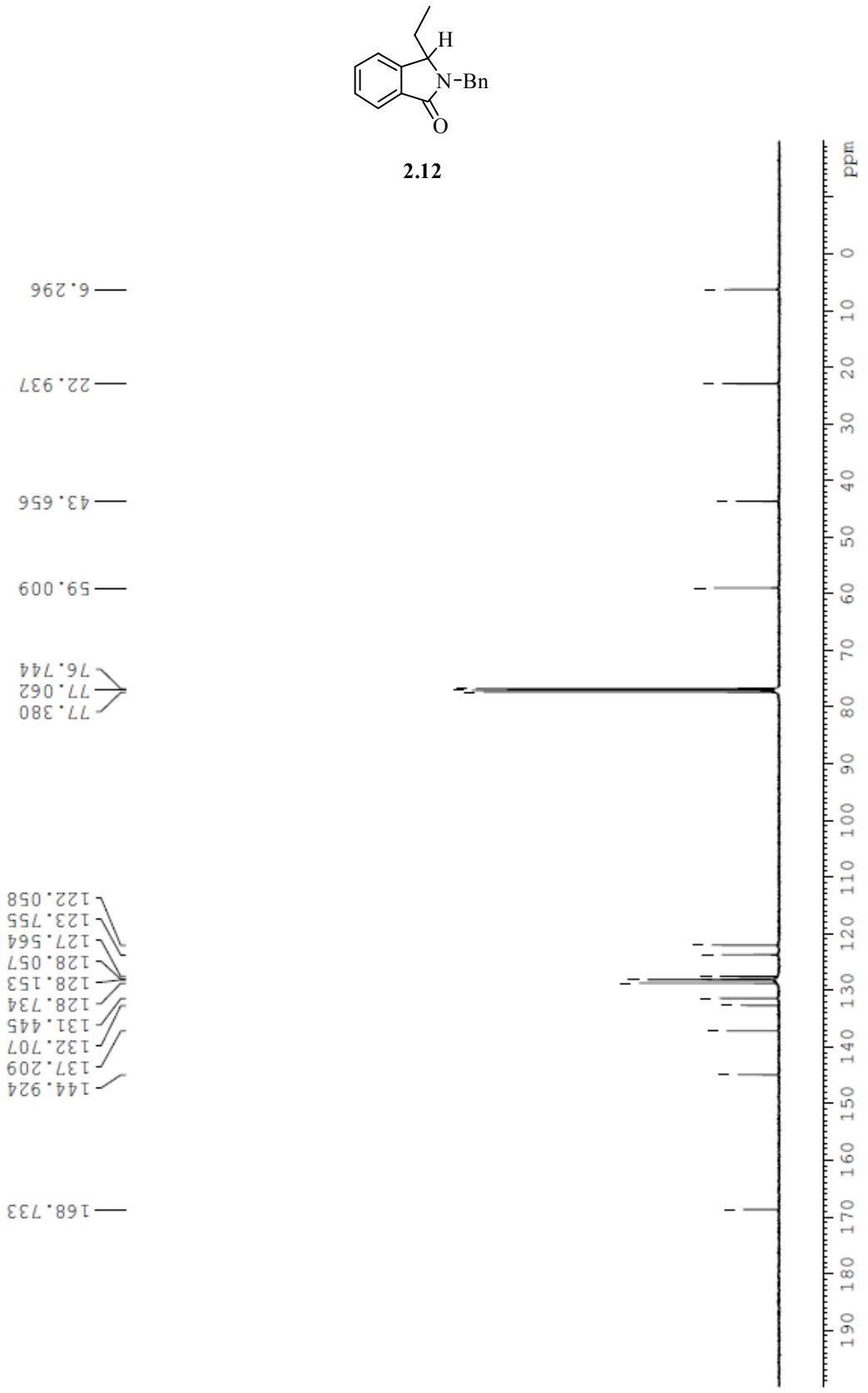
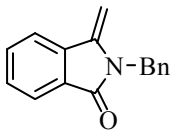


Figure A38: ^{13}C-NMR spectrum of **3.1** ($CDCl_3$)



3.1

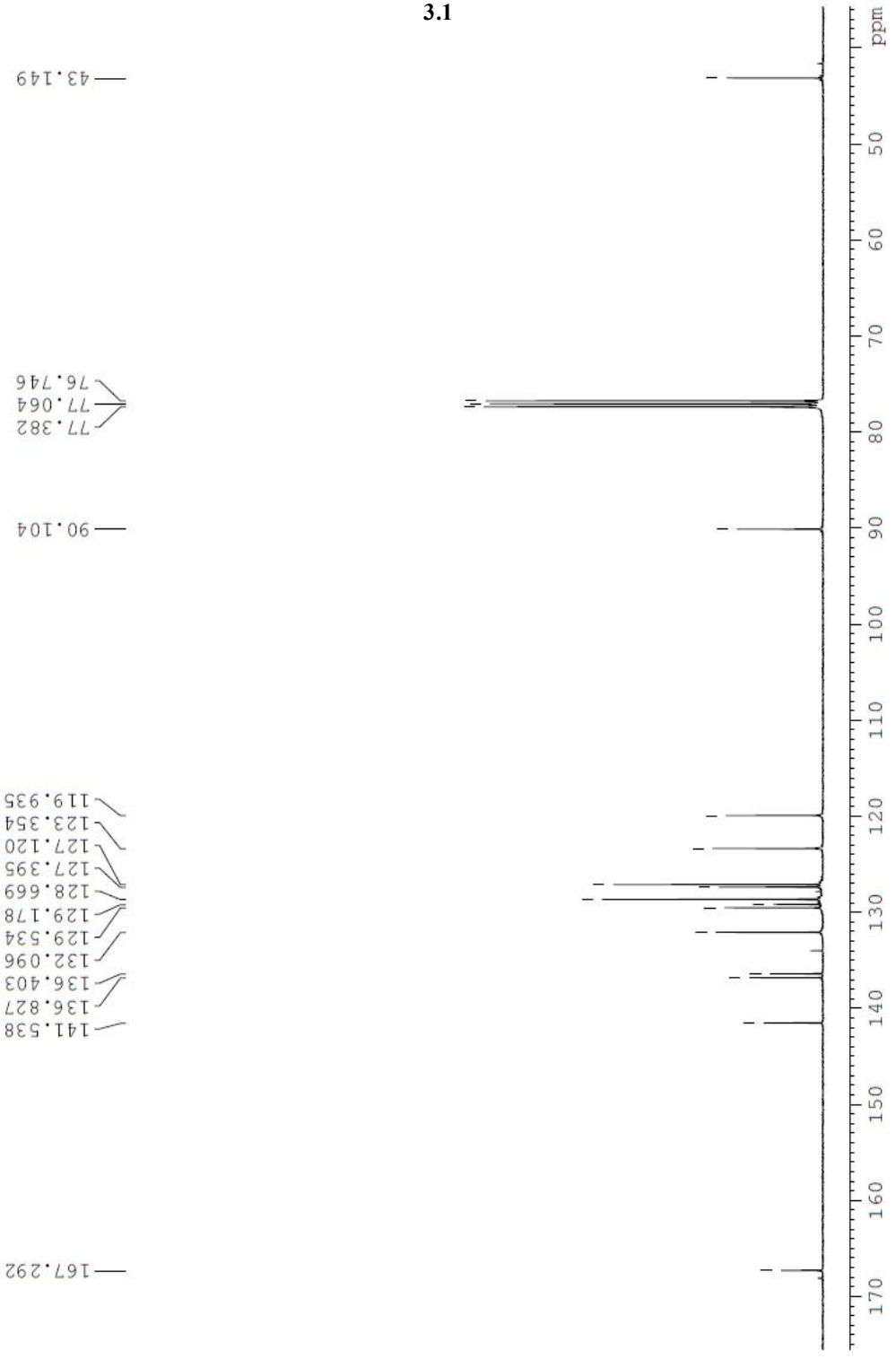


Figure A39: ^{13}C-NMR spectrum of **3.2** ($CDCl_3$)

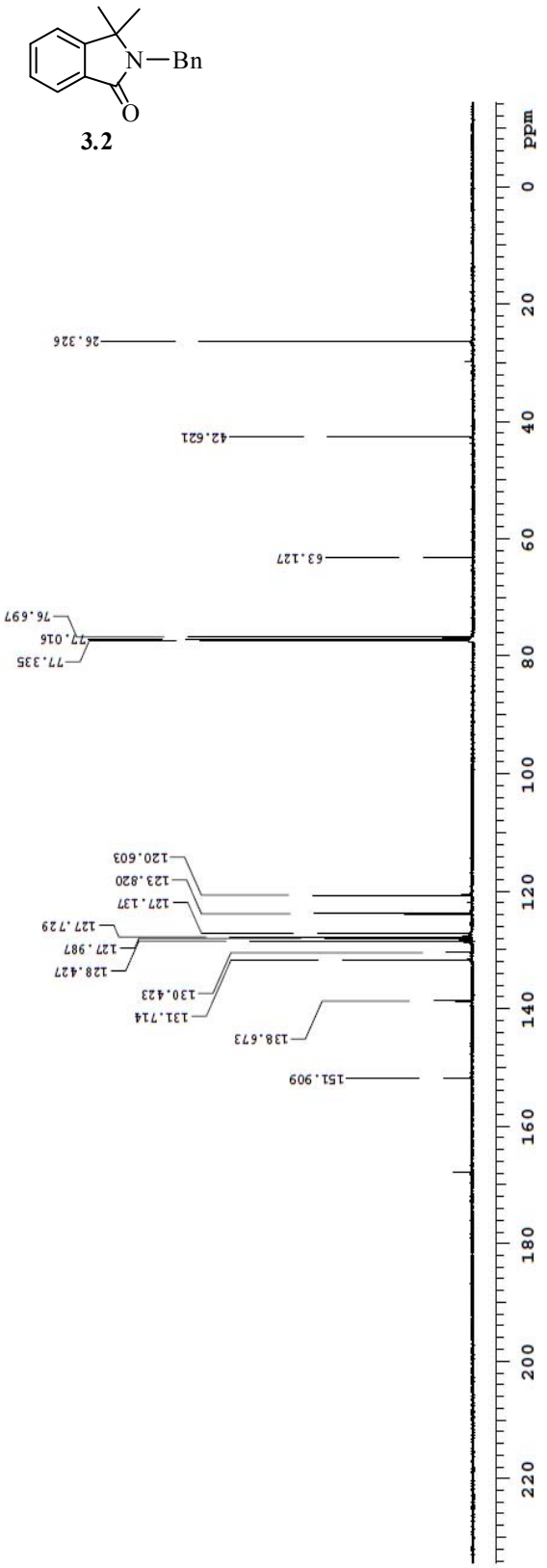


Figure A40: ^{13}C-NMR spectrum of **3.3** ($CDCl_3$)

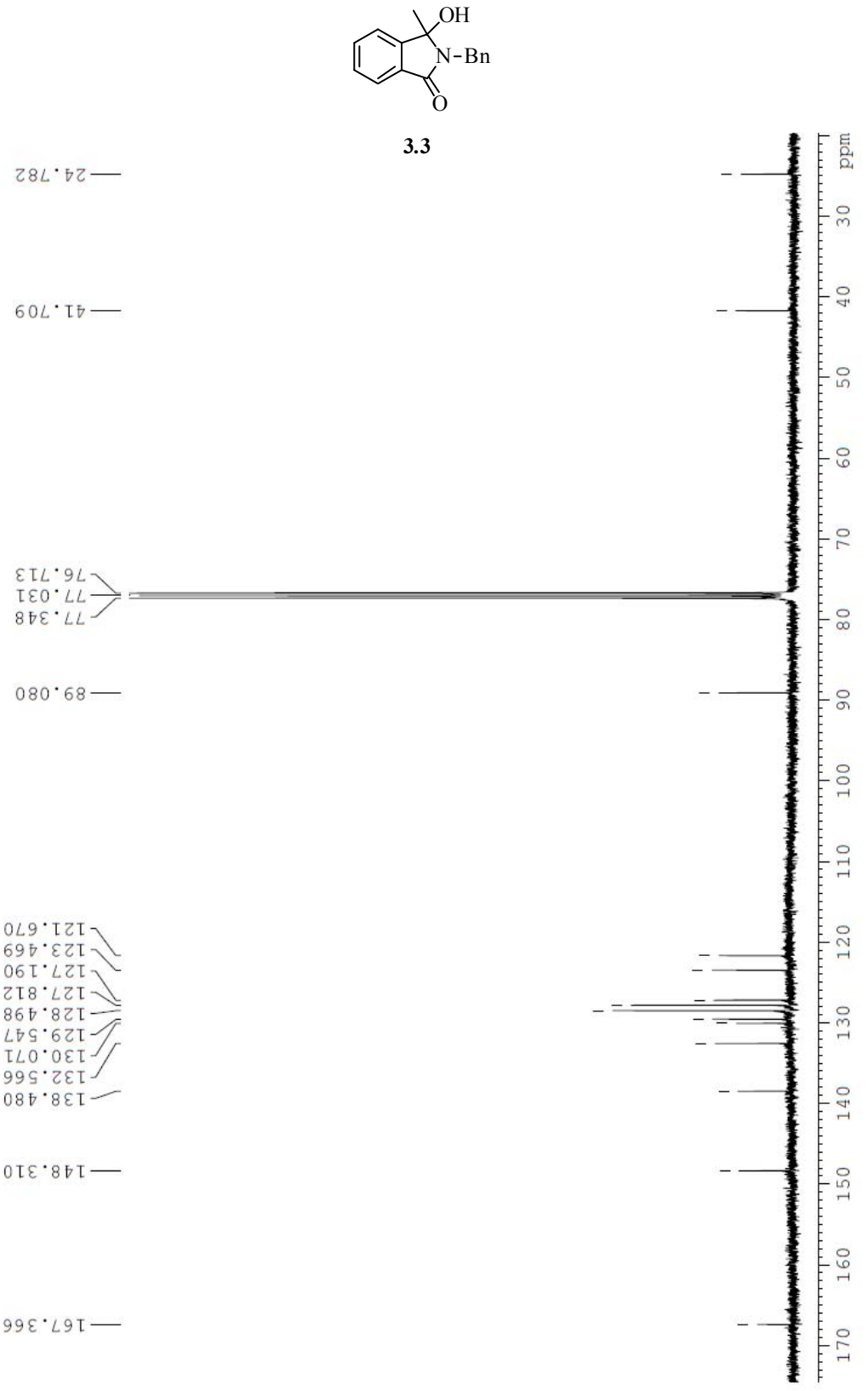


Figure A41: ^{13}C-NMR spectrum of **3.4b** ($CDCl_3$)

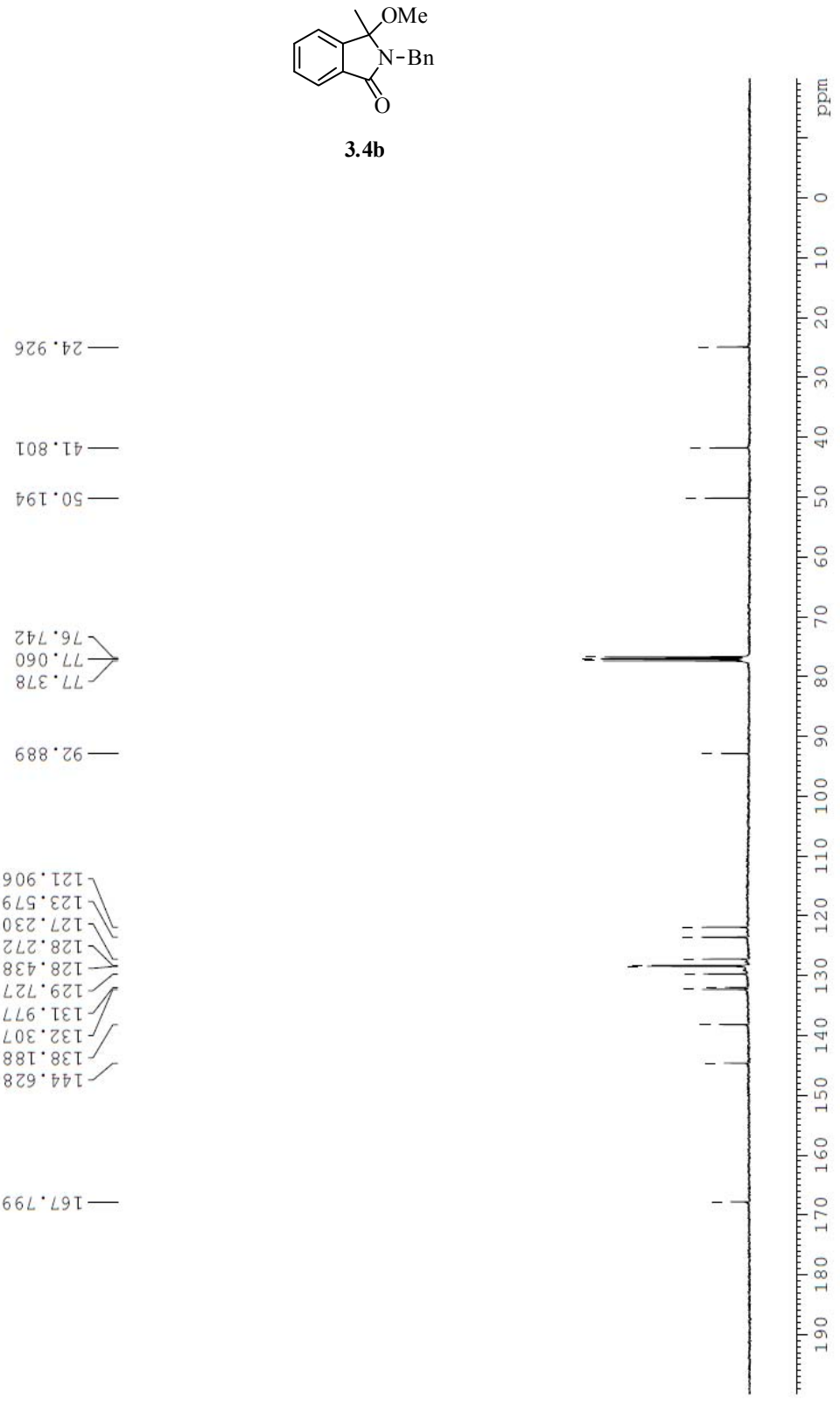


Figure A42: ^{13}C-NMR spectrum of **4.1b** ($CDCl_3$)

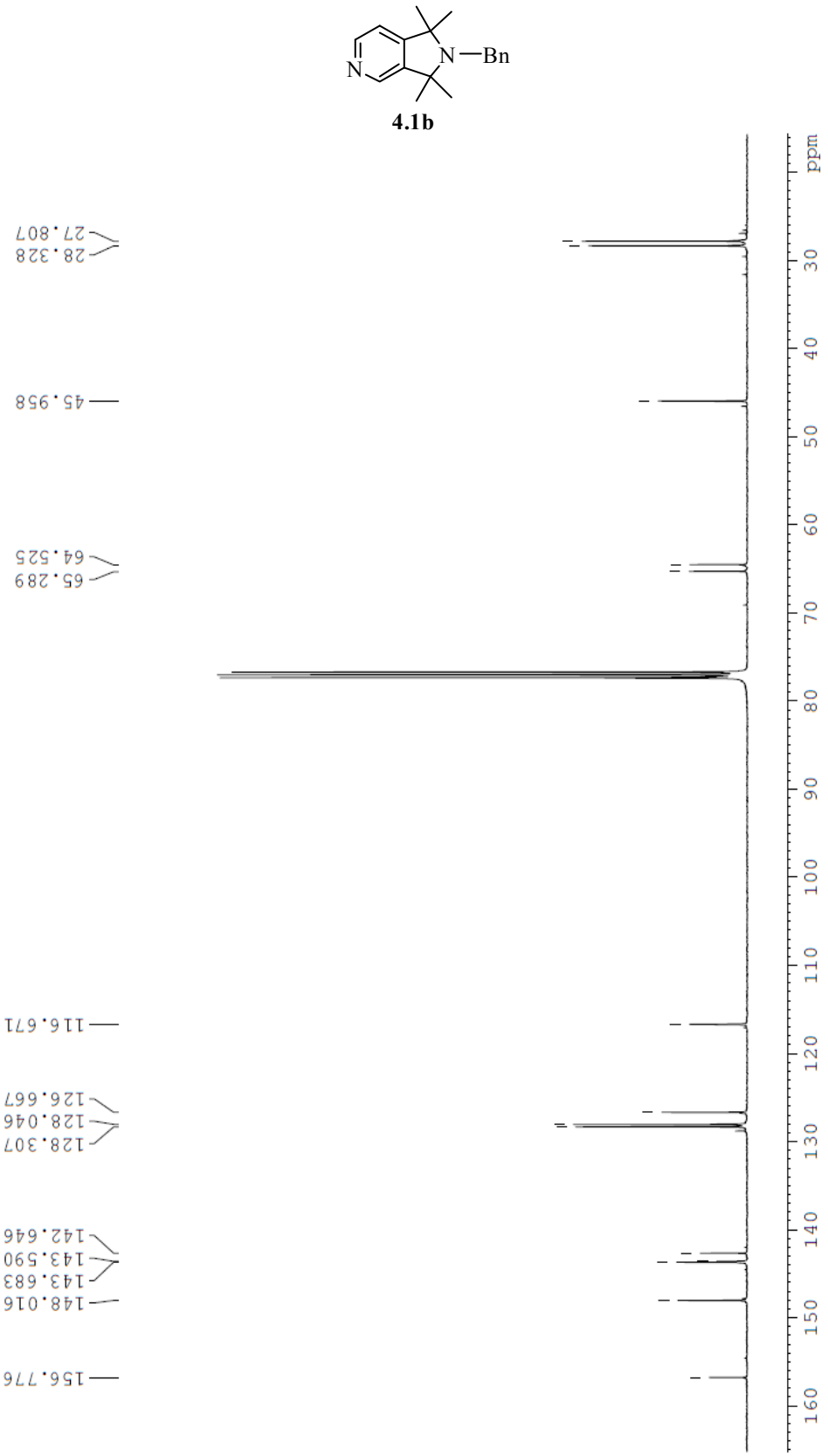


Figure A43: ^{13}C-NMR spectrum of **4.2** ($CDCl_3$)

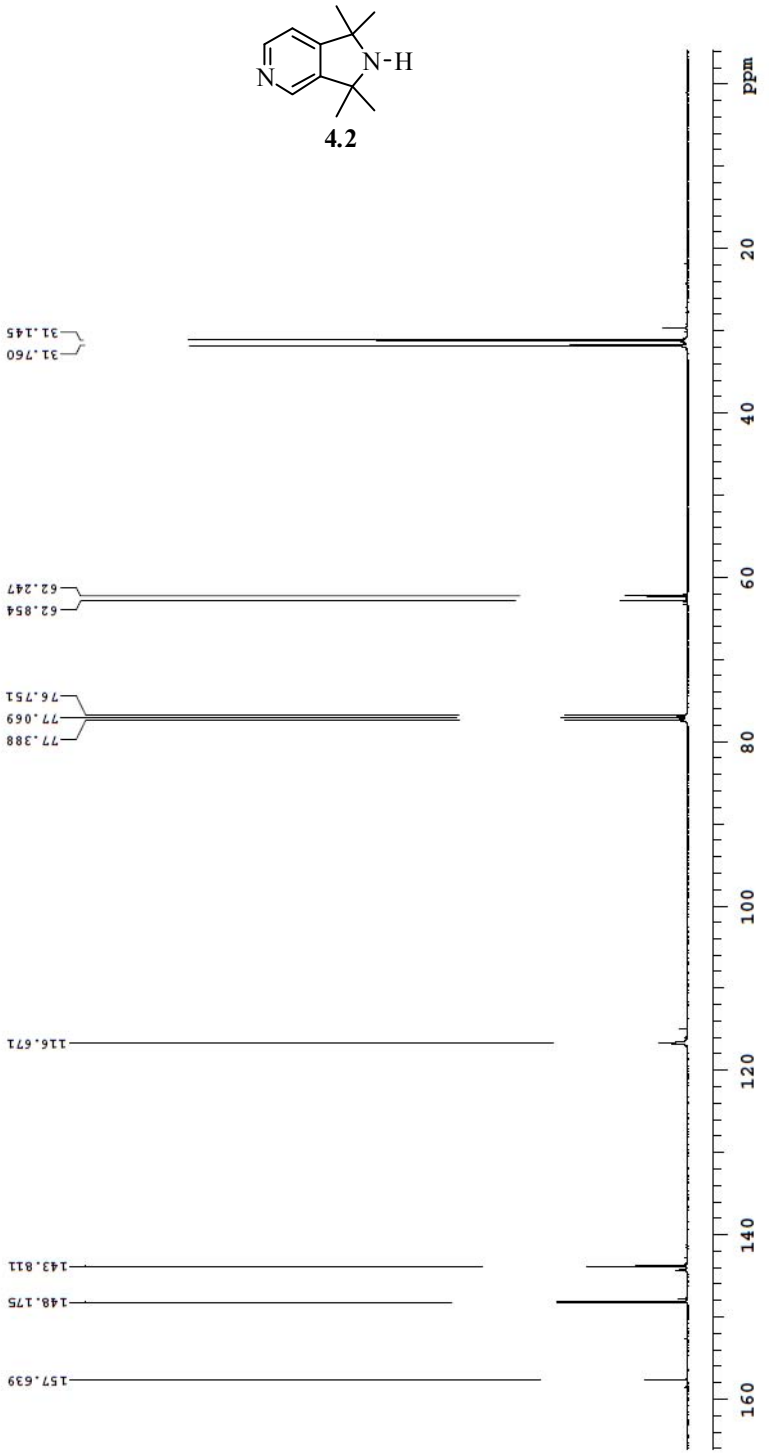


Figure A44: ^{13}C-NMR spectrum of **4.5** ($CDCl_3$)

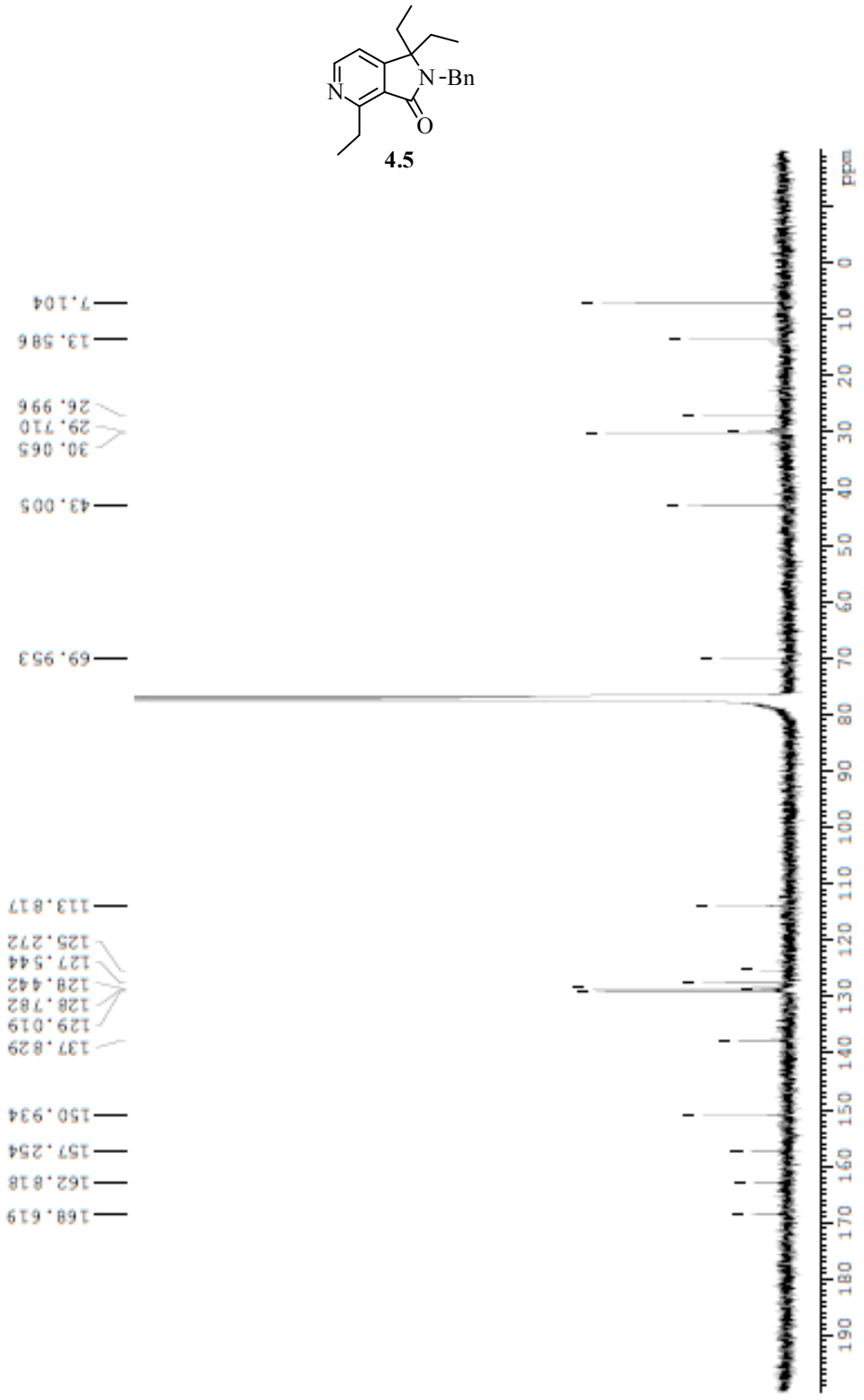
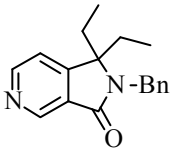


Figure A45: ^{13}C-NMR spectrum of **4.6** ($CDCl_3$)



4.6

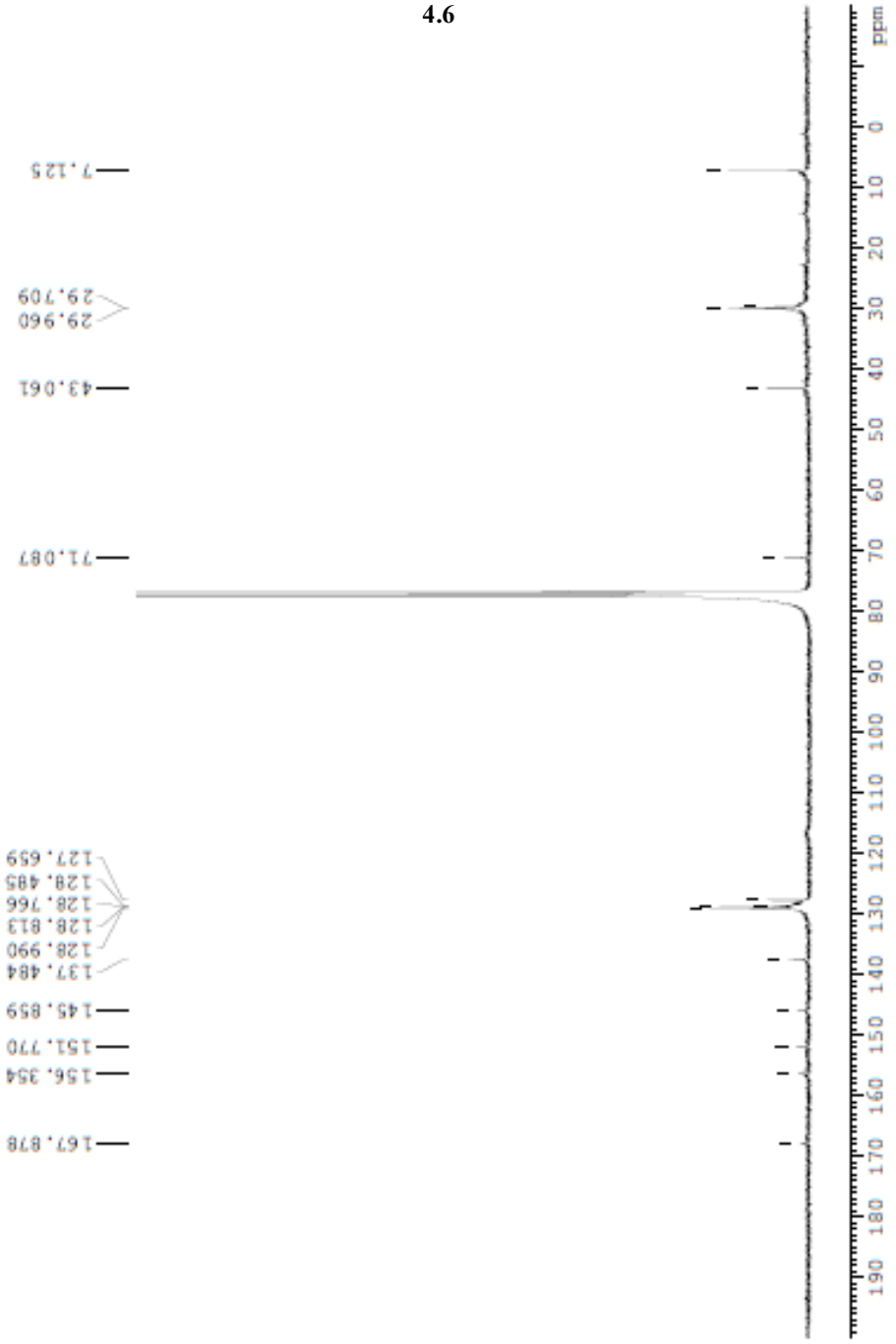
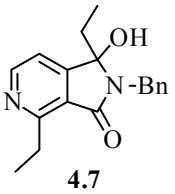


Figure A46: ^{13}C-NMR spectrum of **4.7** ($CDCl_3$)



4.7

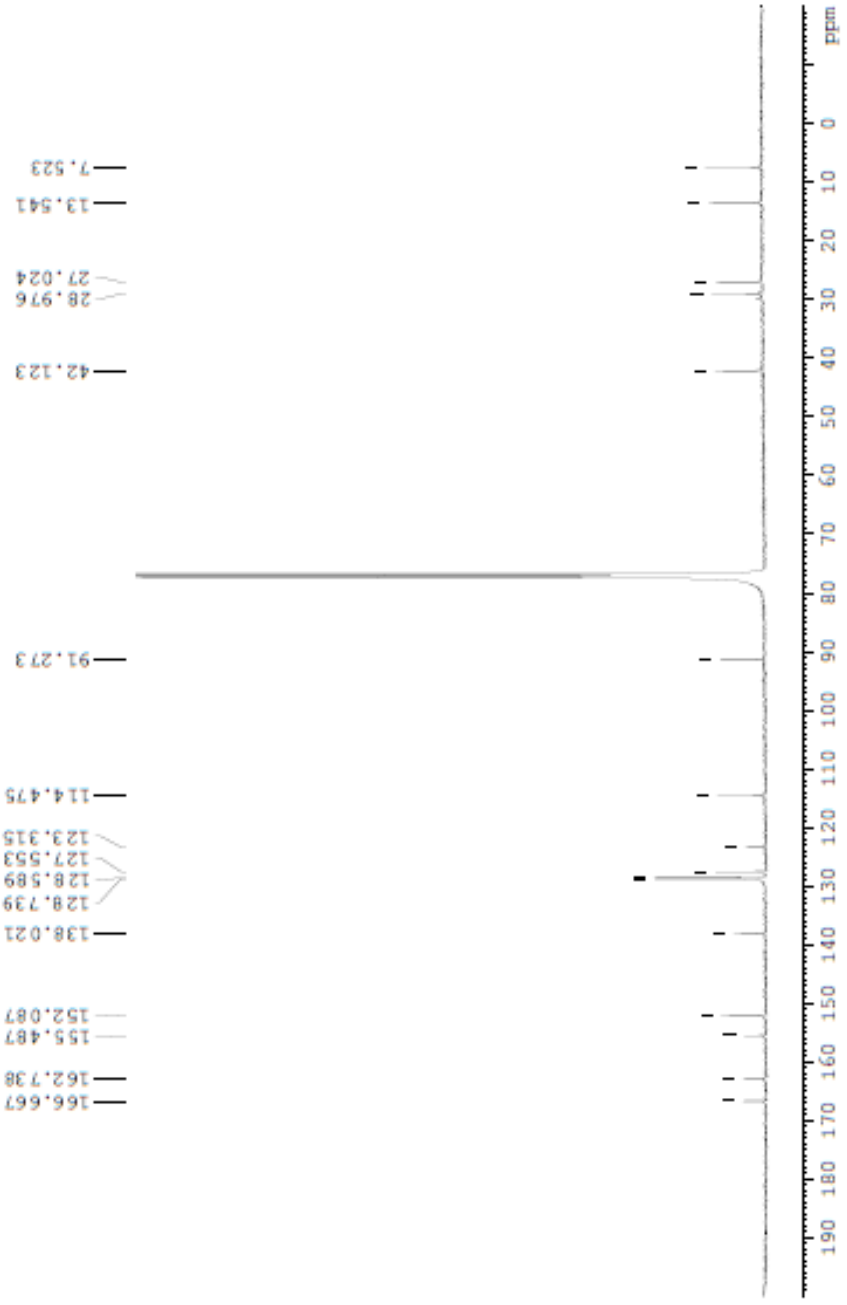
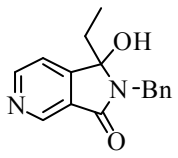


Figure A47: ^{13}C-NMR spectrum of **4.8** ($CDCl_3$)



4.8

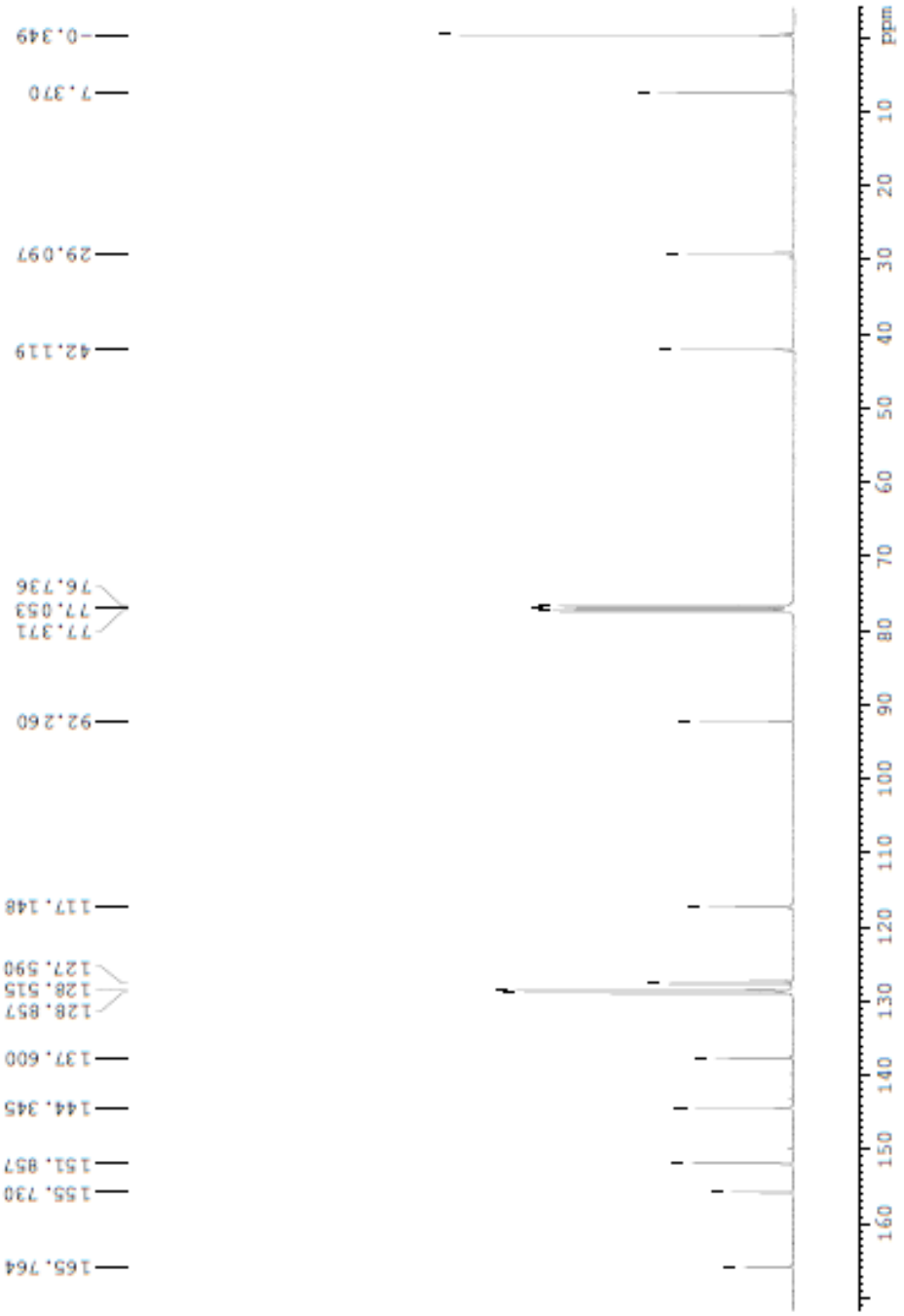


Figure A48: ^{13}C-NMR spectrum of **4.15** ($CDCl_3$)

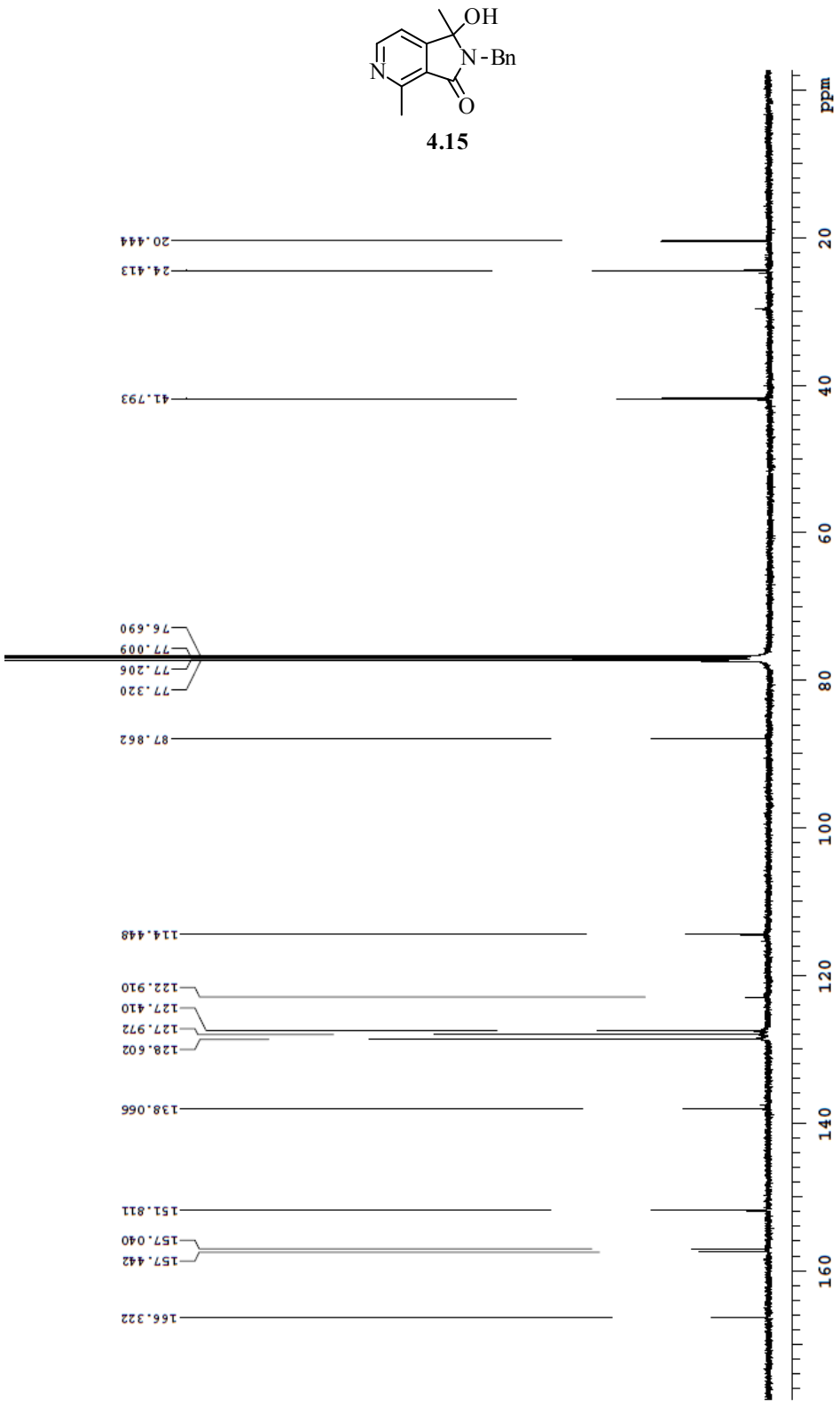


Figure A49: ^{13}C-NMR spectrum of **4.16** ($CDCl_3$)

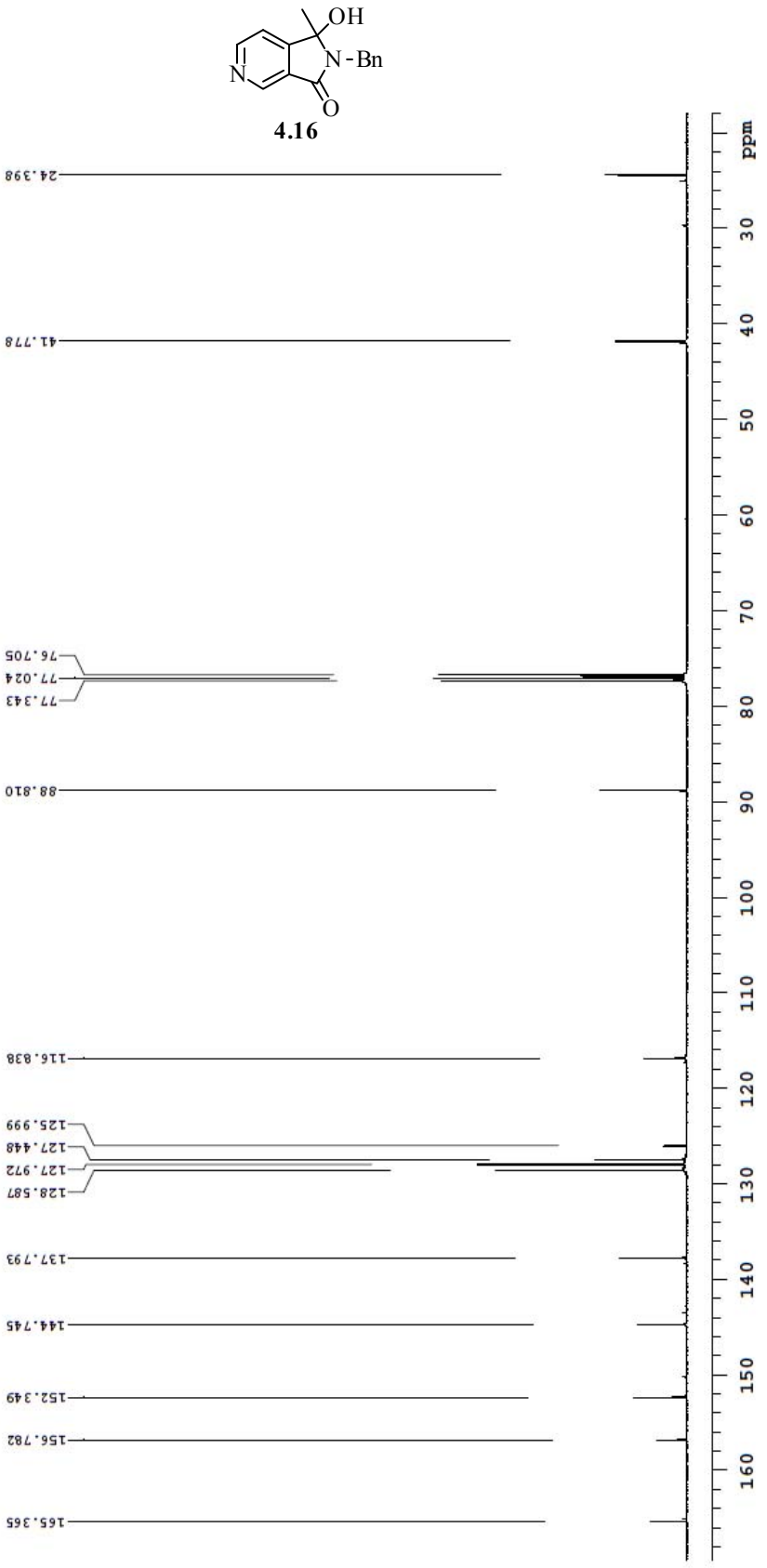
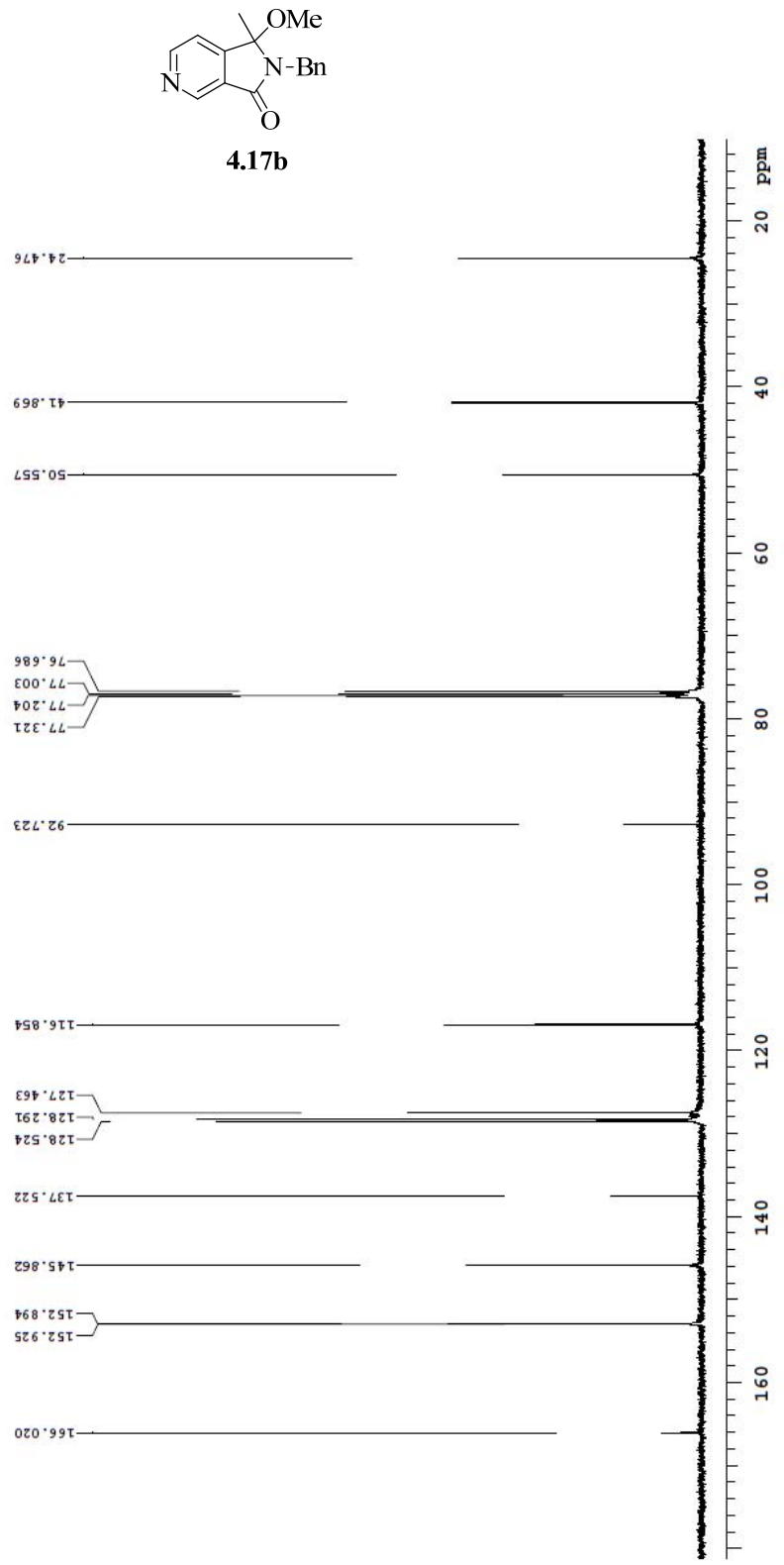


Figure A50: ^{13}C-NMR spectrum of **4.17b** ($CDCl_3$)



8.3 HPLC DATA

(All the HPLC Chromatograms appearing in this section are of **≥95%** purified compounds which were detected at **254 nm** in the stated mixtures of MeOH and H_2O;

HPLC column used in the analysis was

Prep-C18 scalar column (4.6 × 150 mm, 10 μm))

Figure A51. Compound **2.2**, HPLC chromatogram (eluent 65% methanol/35% water)

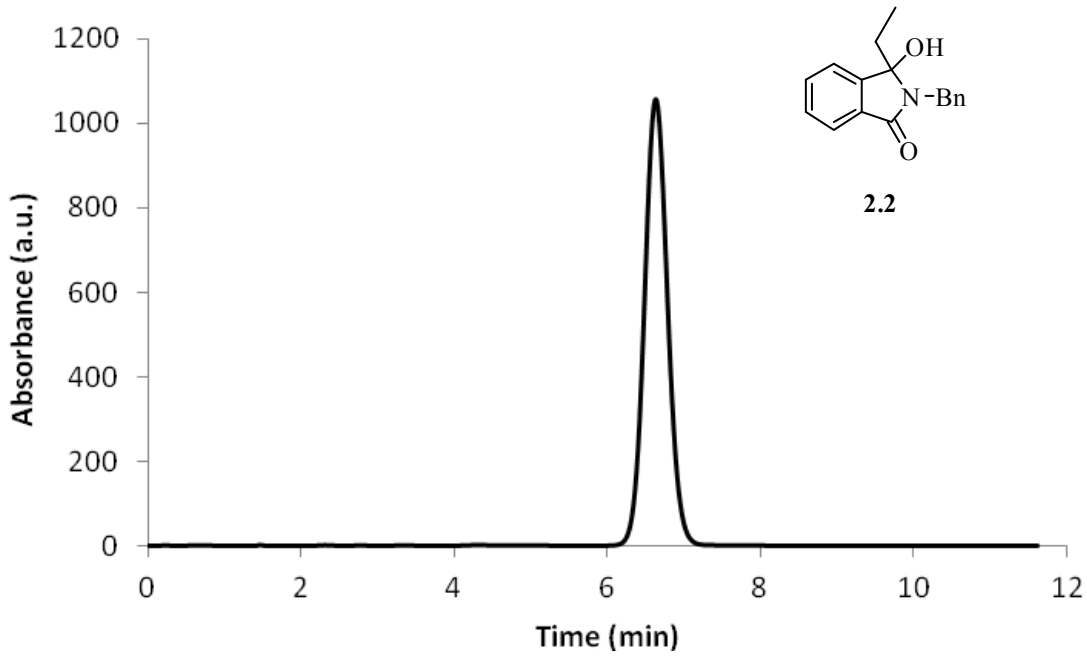


Figure A52. *(E)*-Compound **2.6**, HPLC chromatogram (eluent 65% methanol/35% water).

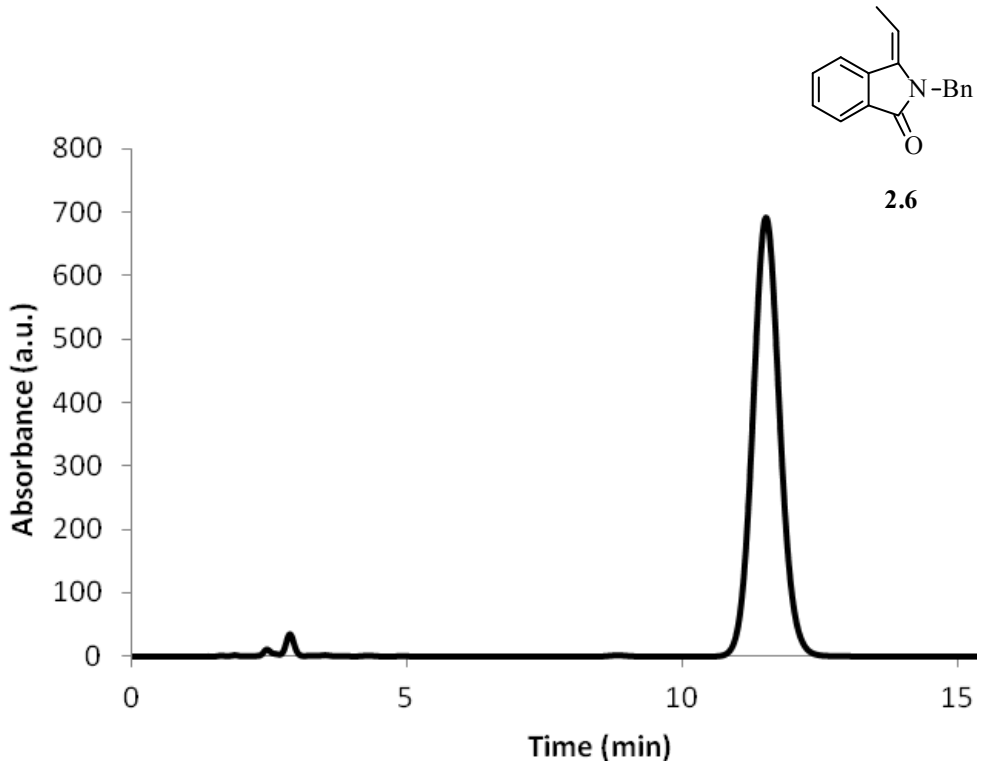


Figure A53. Compound **2.3b**, HPLC chromatogram (eluent 65% methanol/35% water).

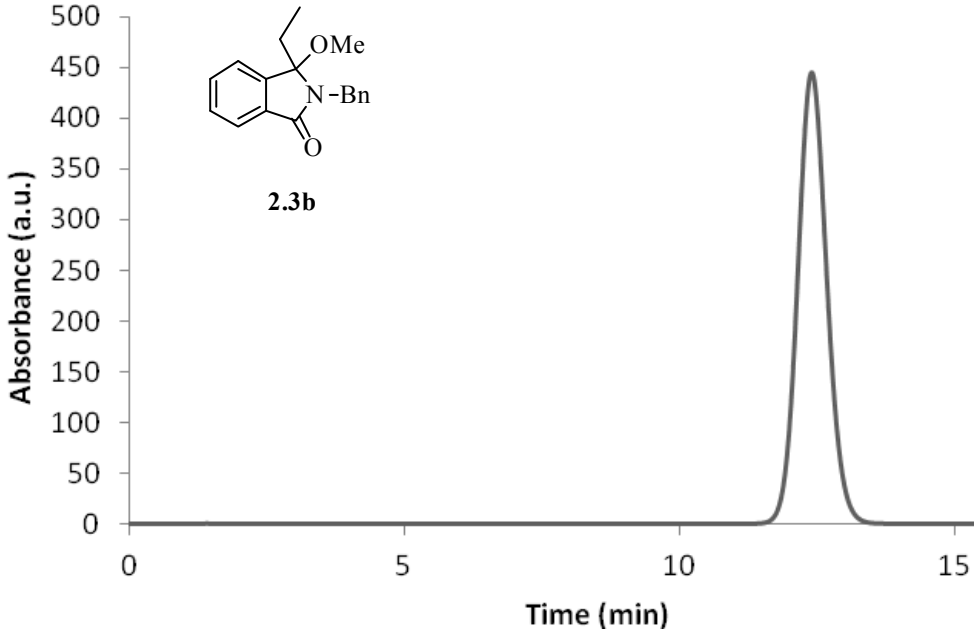


Figure A54. Compound **1.94**, HPLC chromatogram (eluent 65% methanol/35% water for 17 minutes, then ramped to 90% methanol/10% water over 10 minutes, then held at 90% methanol/10% water for 30 minutes).

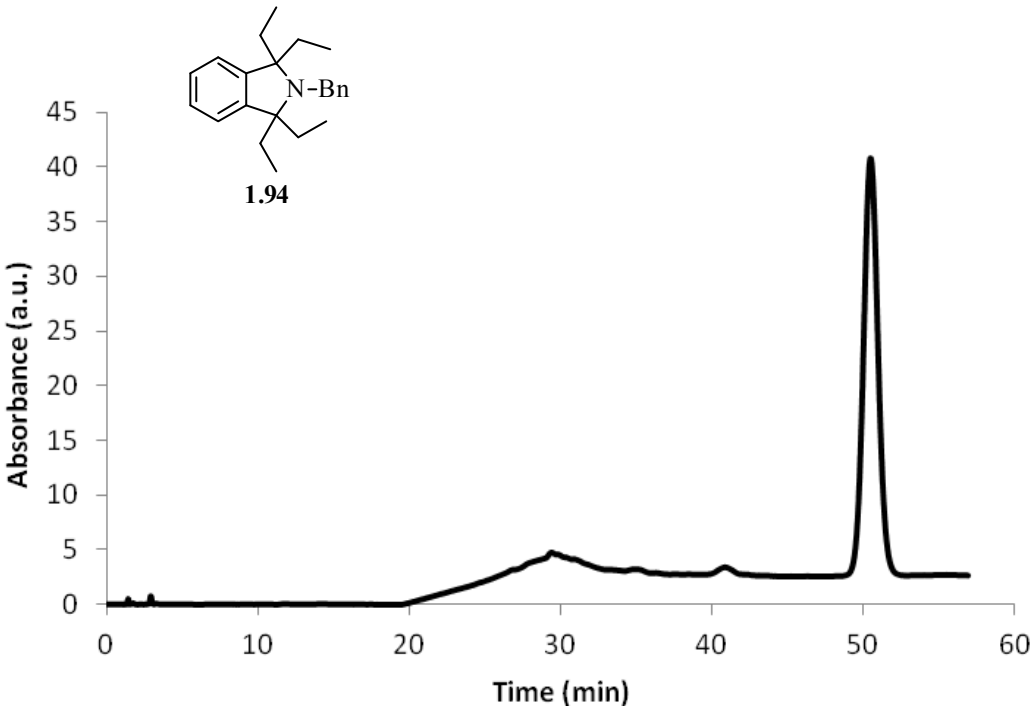


Figure A55. Compound **2.11**, HPLC chromatogram (eluent 65% methanol/35% water).

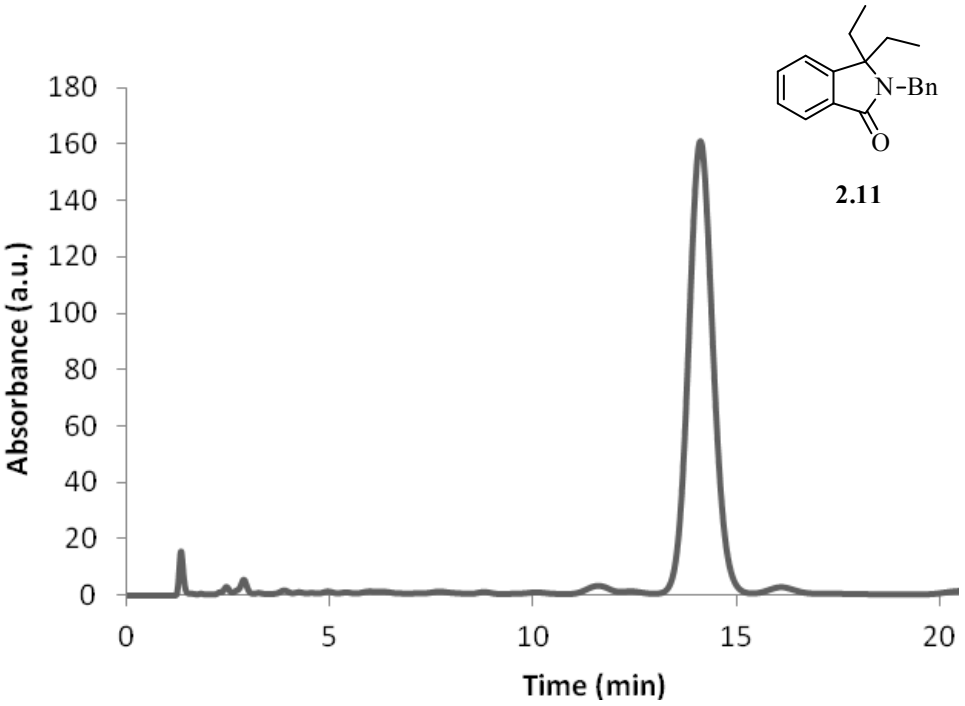


Figure A56. Compound **2.12**, HPLC chromatogram (eluent 65% methanol/35% water).

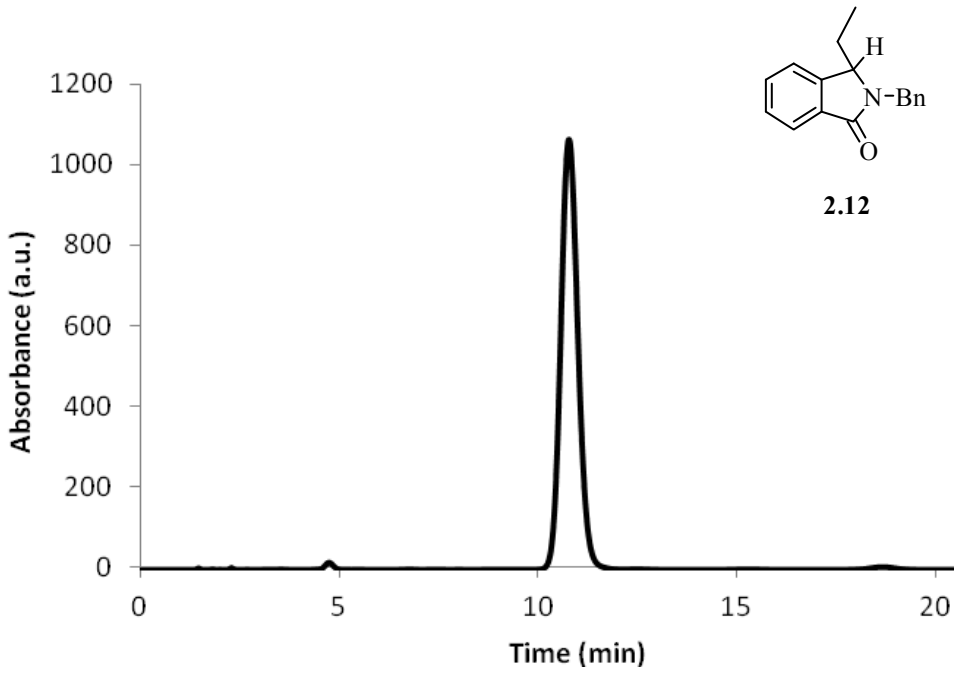


Figure A57. Compound **3.3**, HPLC chromatogram (eluent 65% methanol/35% water).

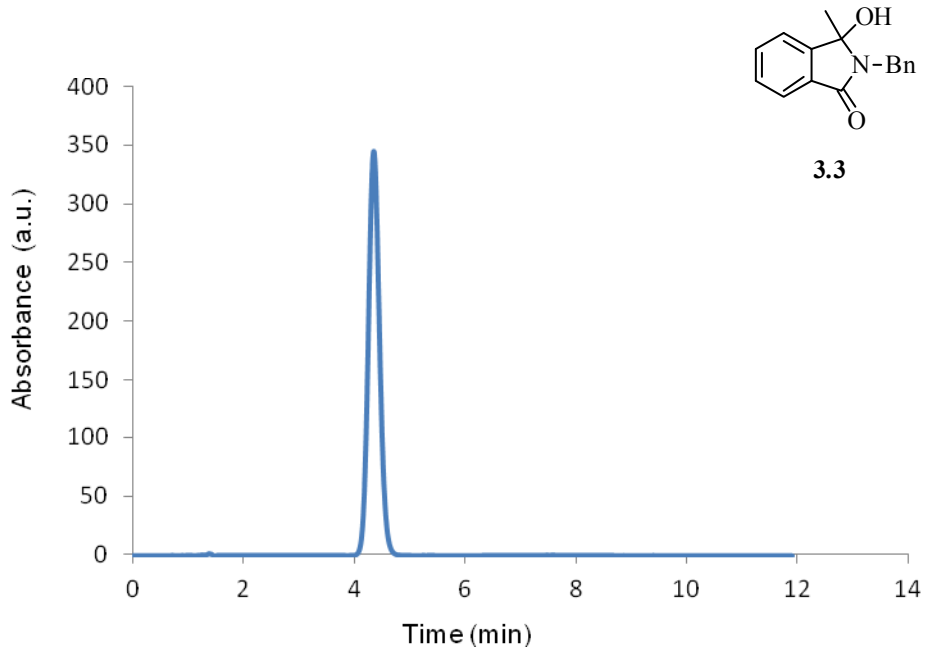


Figure A58. Compound **3.1**, HPLC chromatogram (eluent 65% methanol/35% water).

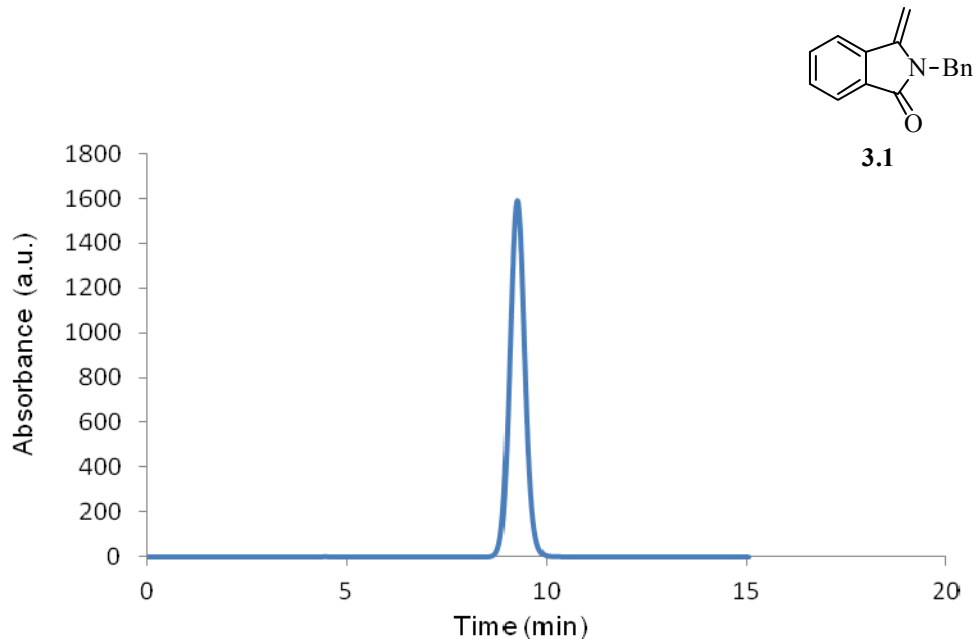


Figure A59. Compound **3.4b**, HPLC chromatogram (eluent 65% methanol/35% water).

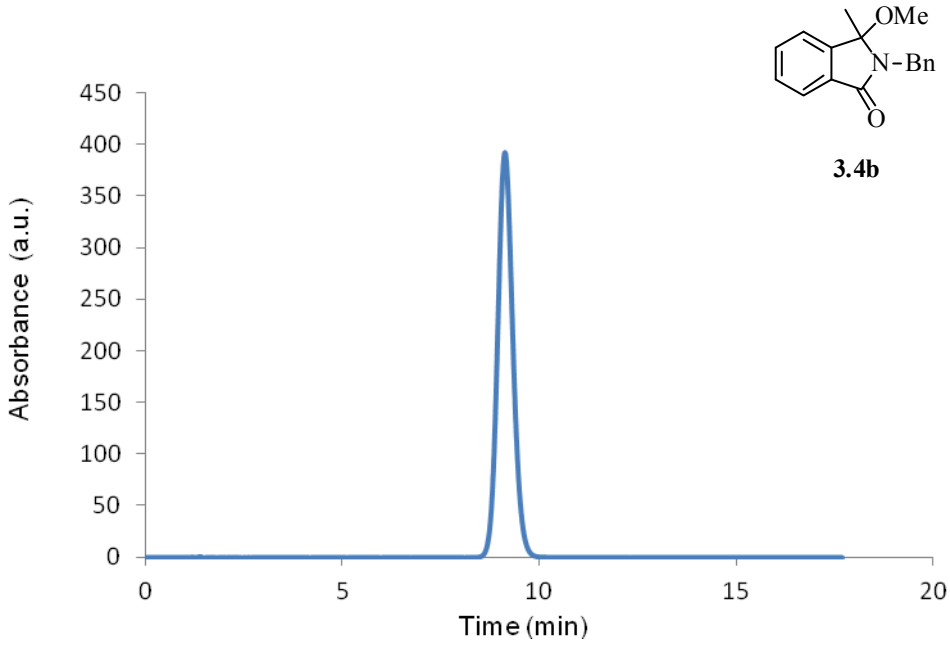


Figure A60. Compound **1.76**, HPLC chromatogram (eluent 65% methanol/35% water for 17 minutes, then ramped to 95% methanol/5% water over 10 minutes, then held at 95% methanol/5% water for 15 minutes)

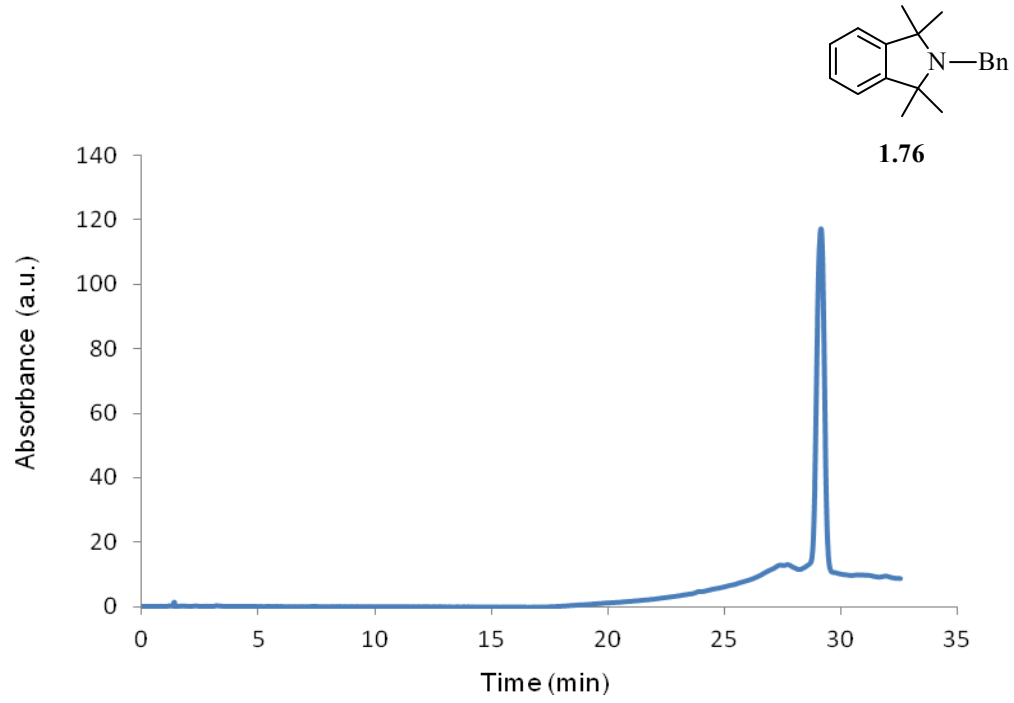


Figure A61. Compound **3.2**, HPLC chromatogram (eluent 65% methanol/35% water).

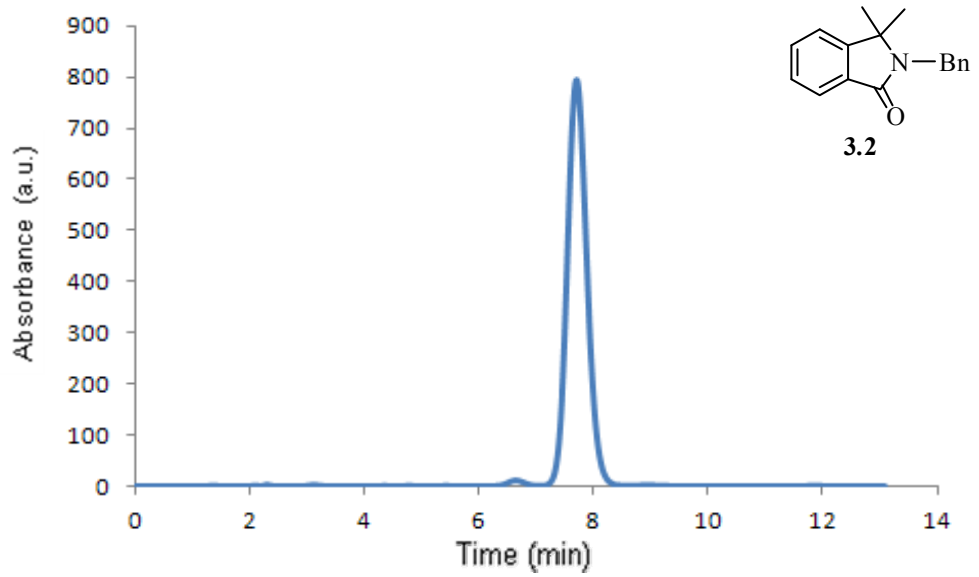


Figure A62. Compound **1.82**, HPLC chromatogram (eluent 65% methanol/35% water for 17 minutes, then ramped to 95% methanol/5% water over 10 minutes, then held at 95% methanol/5% water for 15 minutes)

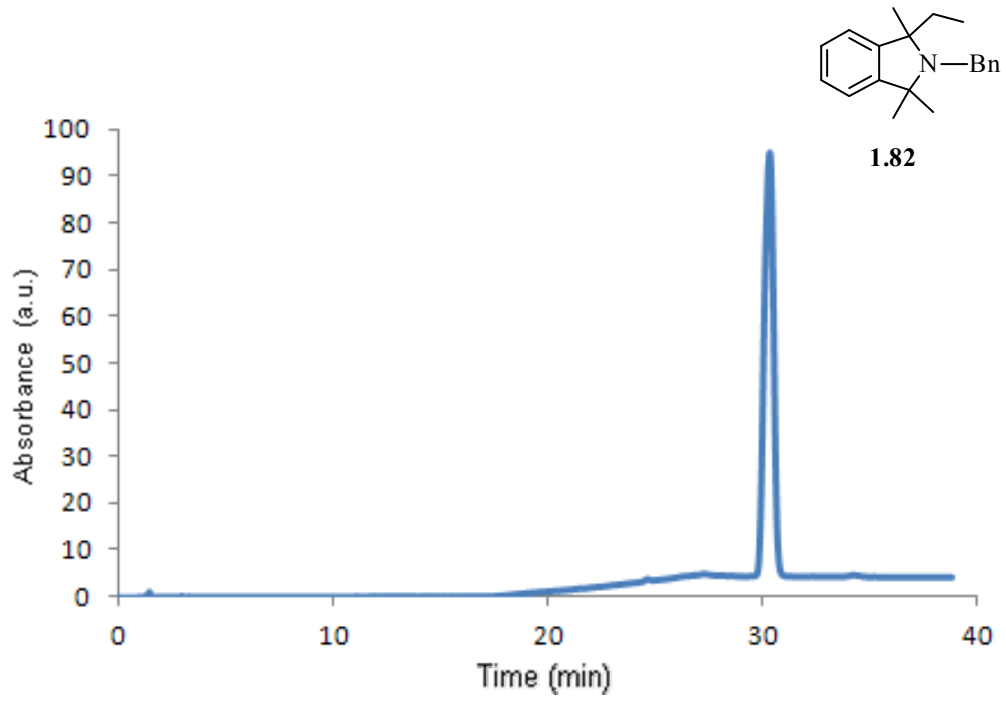


Figure A63. Compound **1.109**, HPLC chromatogram (eluent 65% methanol/35% water)

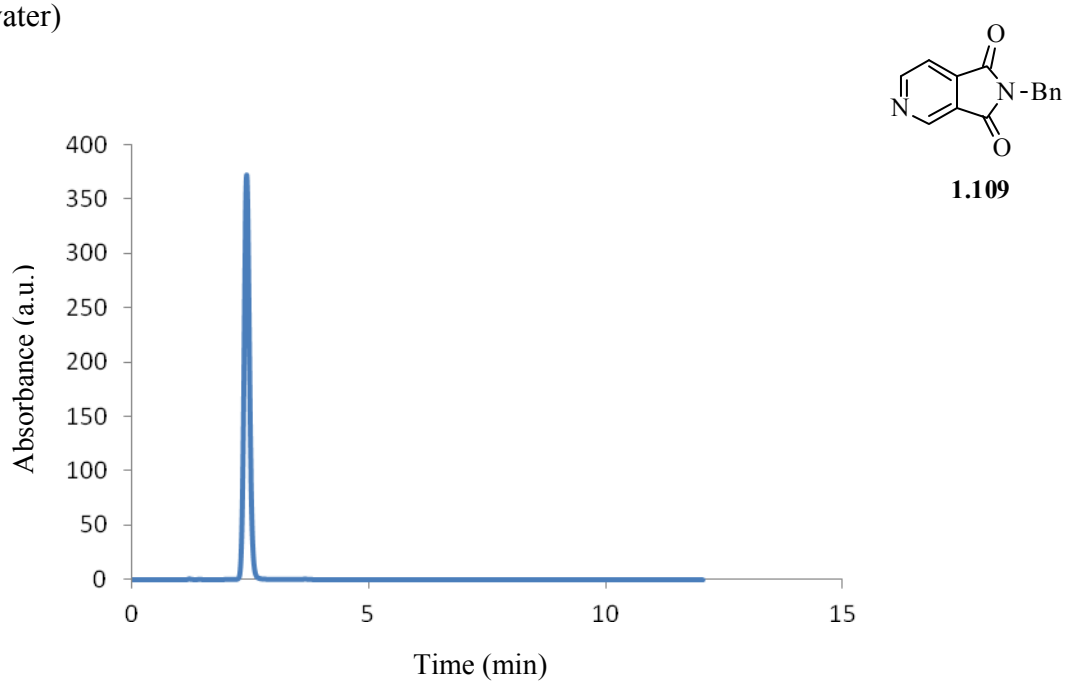


Figure A64. Compound **4.4**, HPLC chromatogram (eluent 65% methanol/35% water)

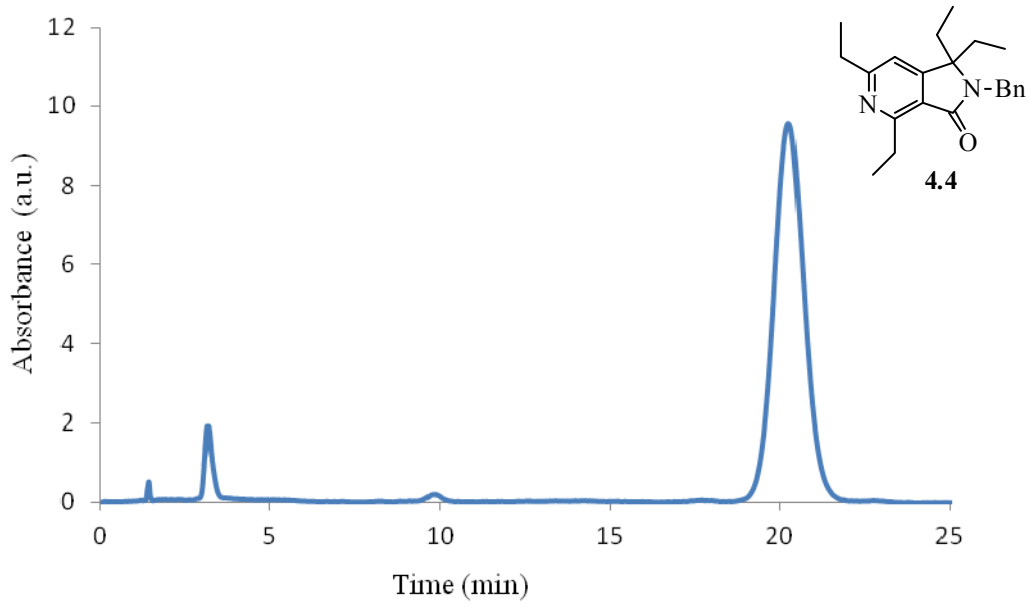


Figure A65. Compound **4.5**, HPLC chromatogram (eluent 65% methanol/35% water)

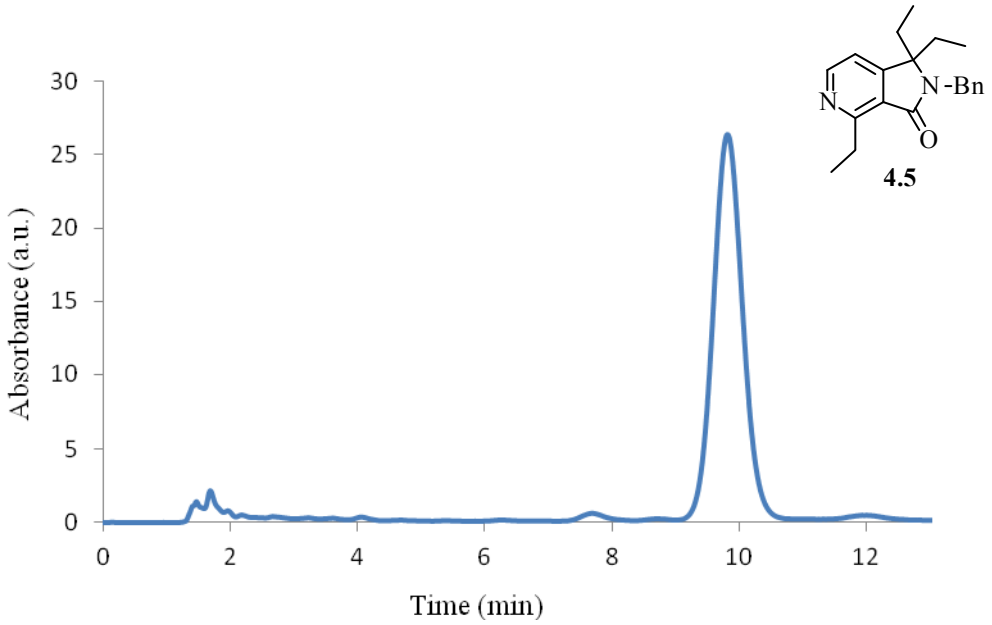


Figure A66. Compound **4.6**, HPLC chromatogram (eluent 65% methanol/35% water)

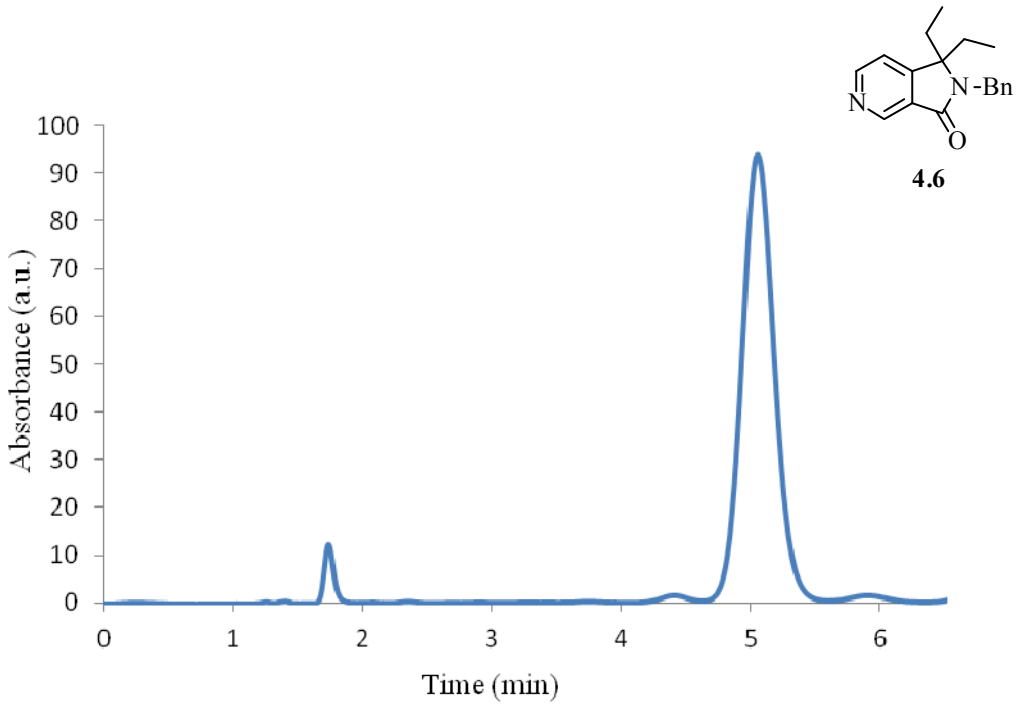


Figure A67. Compound **4.7**, HPLC chromatogram (eluent 65% methanol/35% water)

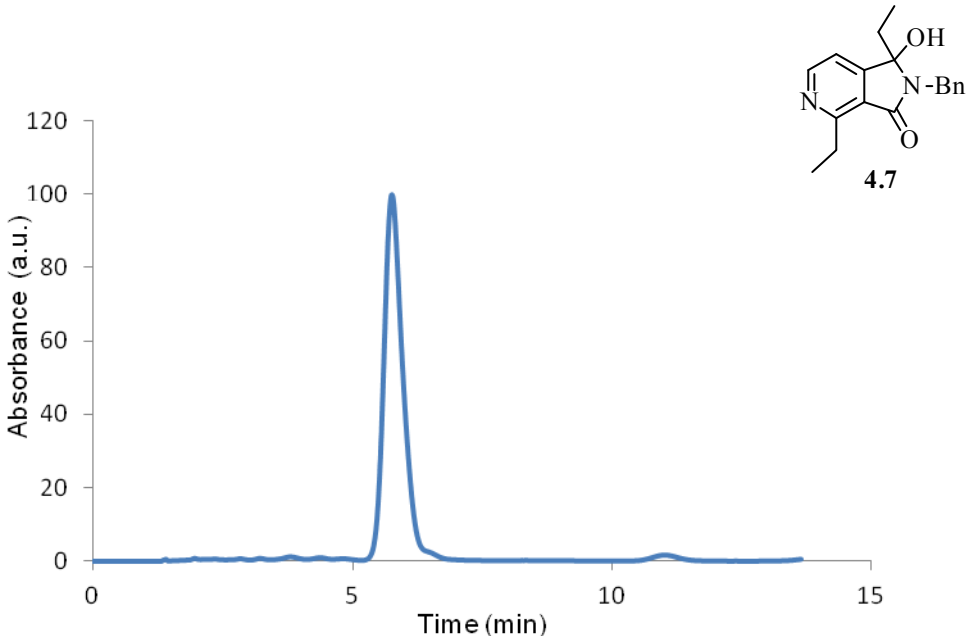


Figure A68. Compound **4.8**, HPLC chromatogram (eluent 65% methanol/35% water)

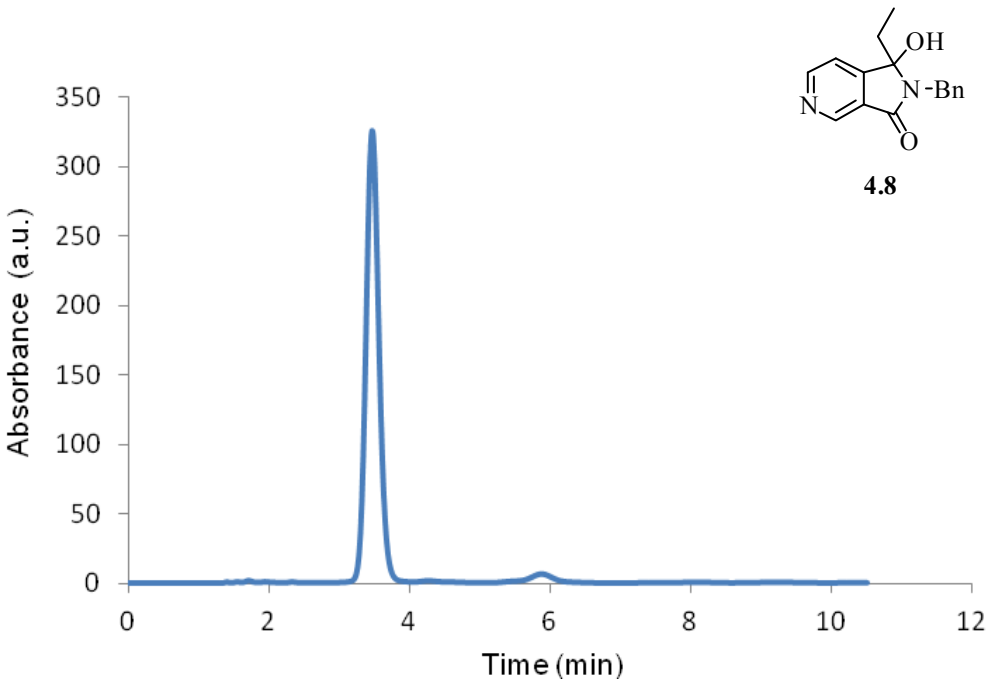


Figure A69. Compound **4.1b**, HPLC chromatogram (eluent 65% methanol/35% water for 15 minutes, then ramped to 90% methanol/10% water over 10 minutes, then held at 90% methanol/10% water for 10 minutes)

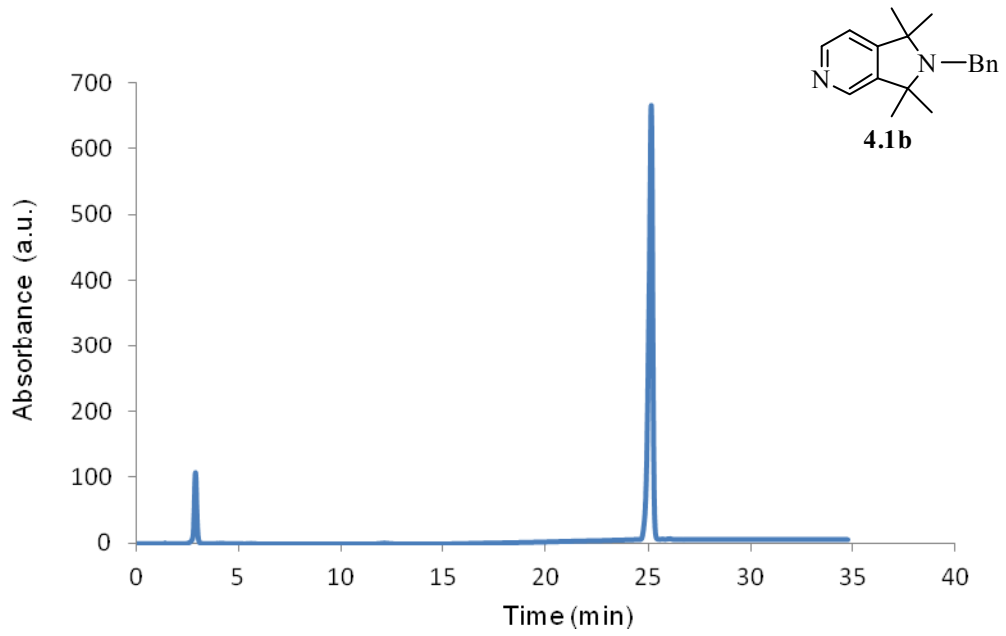


Figure A70. Compound **4.15**, HPLC chromatogram (eluent 65% methanol/35% water)

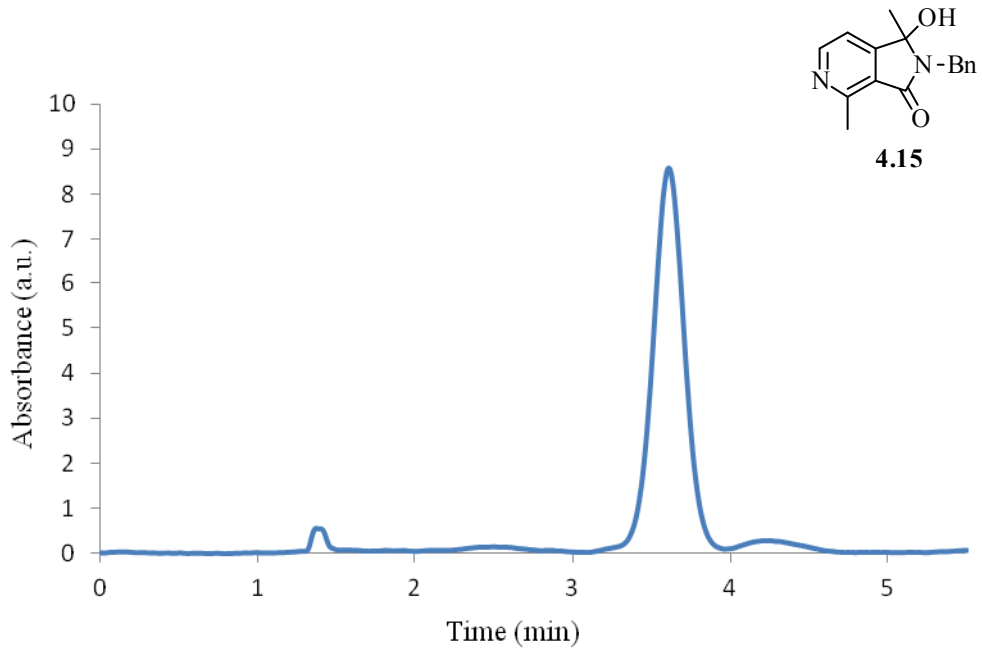


Figure A71. Compound **4.16**, HPLC chromatogram (eluent 65% methanol/35% water)

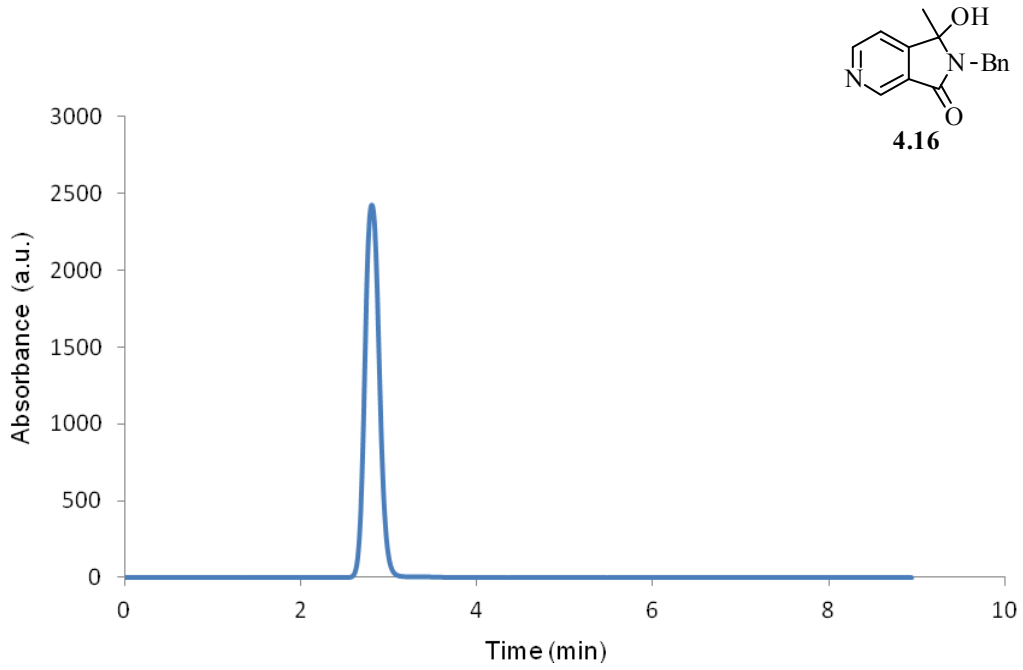


Figure A72. Compound **4.2**, HPLC chromatogram (eluent 70% methanol/30% water)

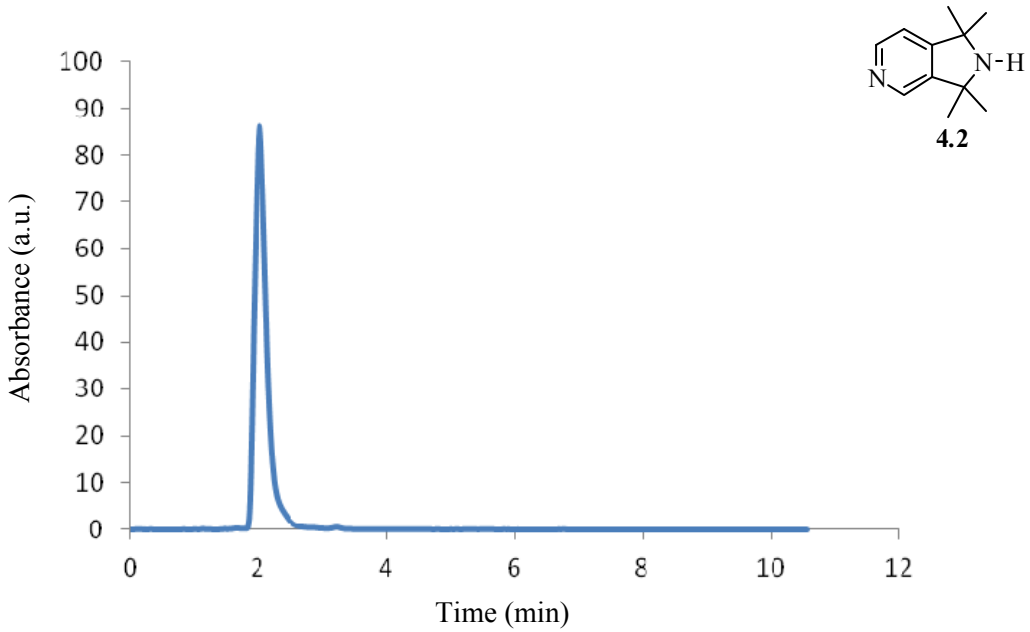


Figure A73. Compound **4.3**, HPLC chromatogram (eluent 70% methanol/30% water)

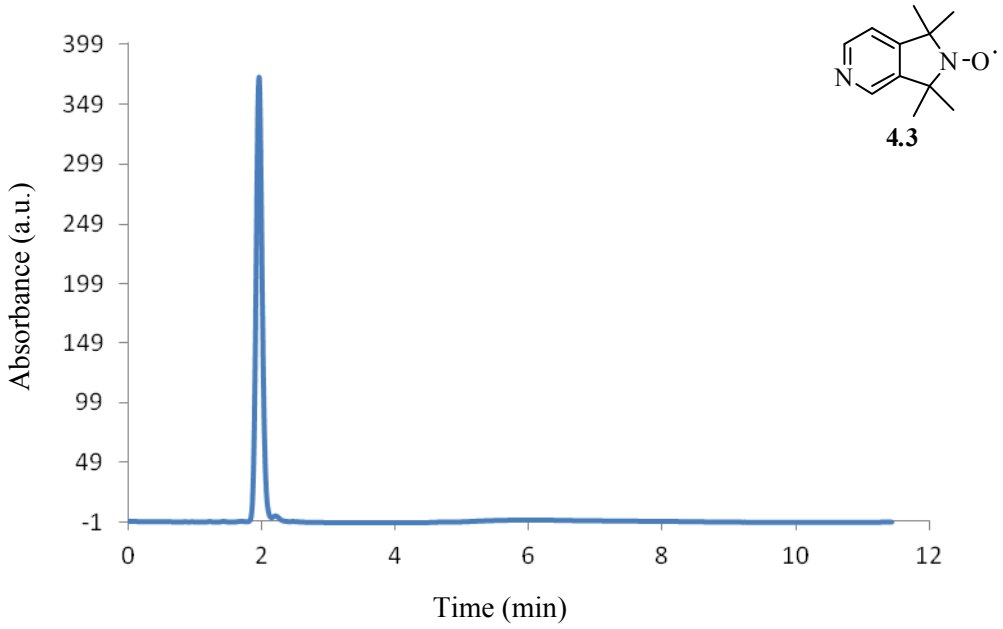
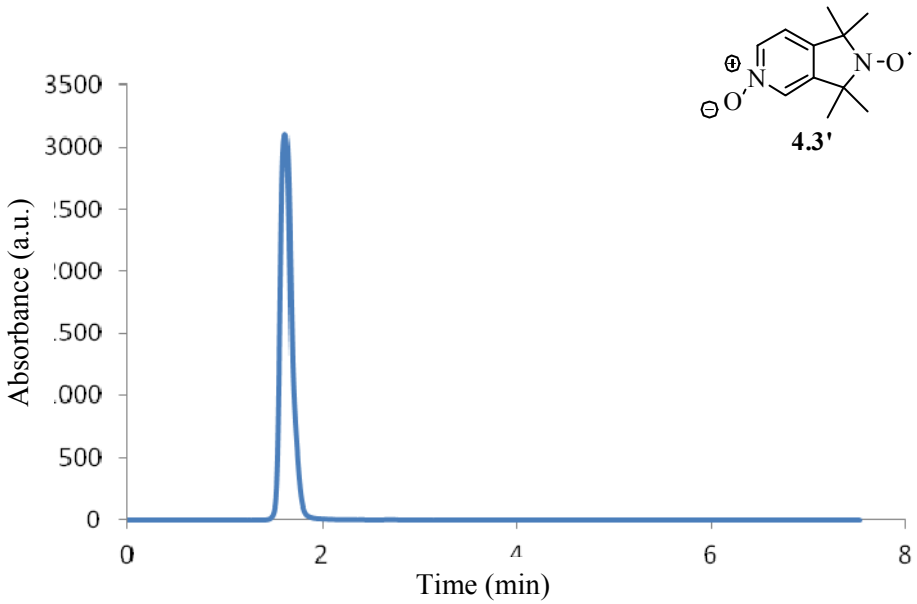


Figure A74. Compound **4.3'**, HPLC chromatogram (eluent 70% methanol/30% water)



8.4 EPR DATA

Figure A75. Compound **4.3**, EPR spectrum (100% MeOH).

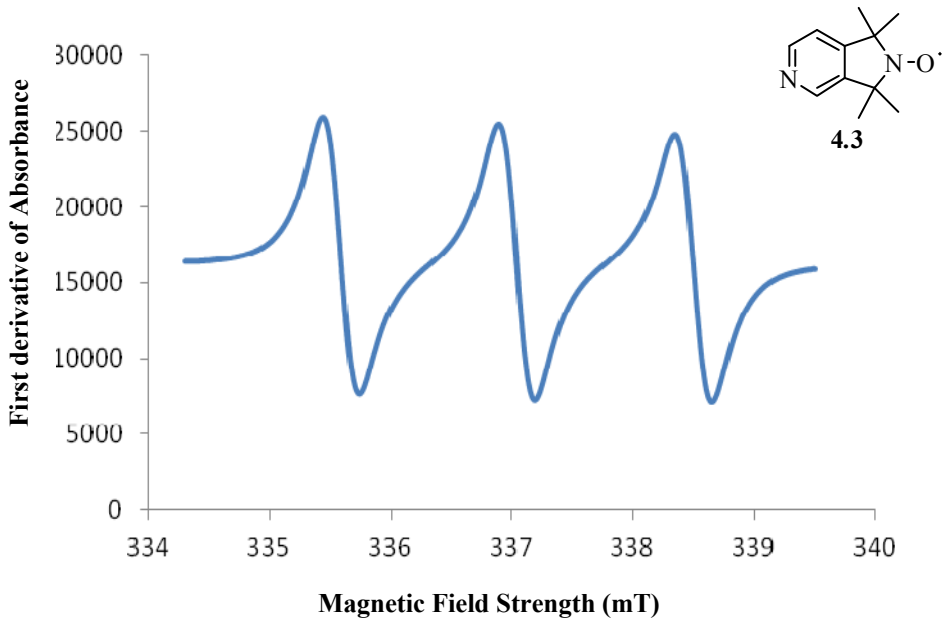
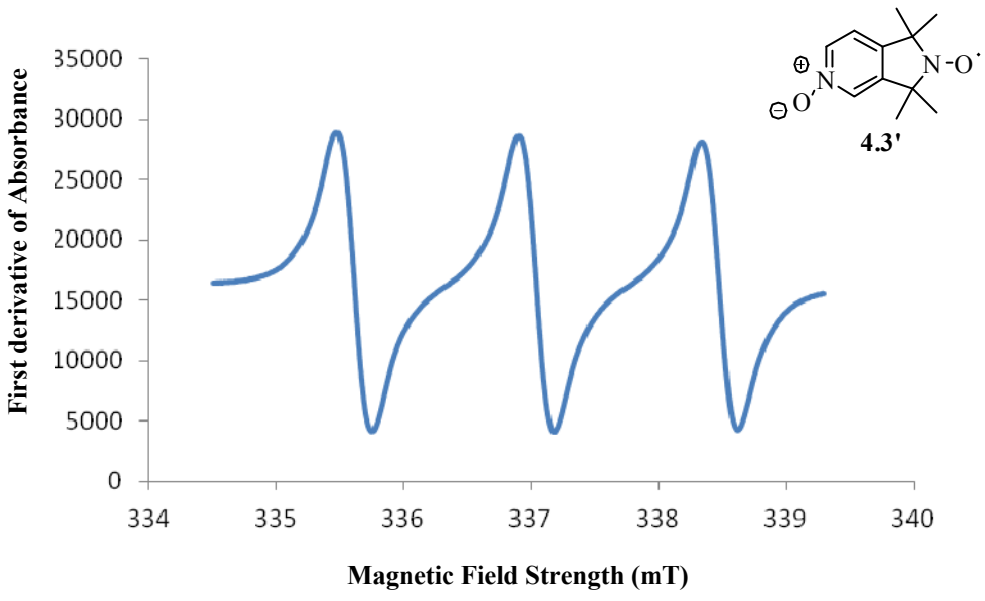


Figure A76. Compound **4.3'**, EPR spectrum (100% MeOH).

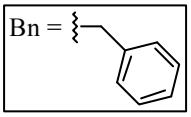


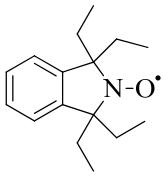
8.5 FIRST PUBLICATION

8.6 LIST OF STRUCTURES THAT APPEAR IN THE THESIS

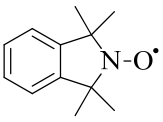
(*): New compounds synthesised during the project

(**): Previously synthesised, but **not fully characterised**.

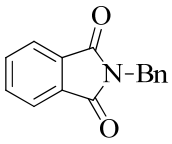




1.13



1.15



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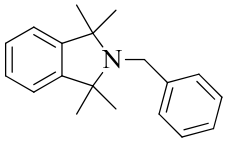
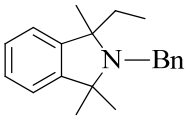
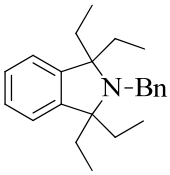


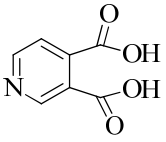
1.76



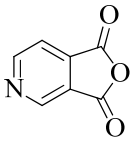
1.82



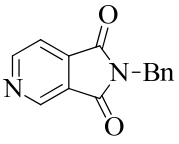
1.94



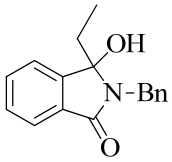
1.106



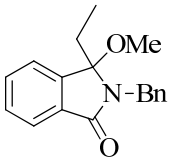
1.108



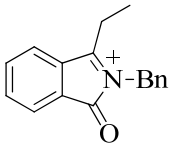
1.109**



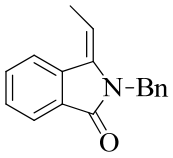
2.2**



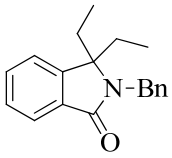
2.3b*



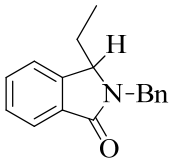
2.5



2.6**



2.11*



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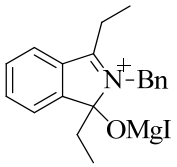

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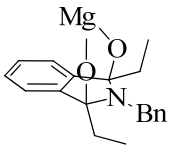

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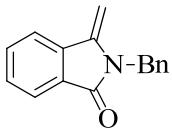
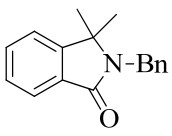


3.1



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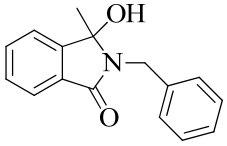

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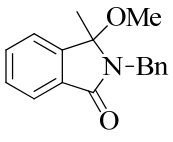

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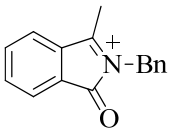

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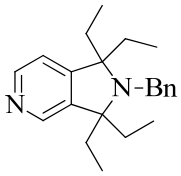

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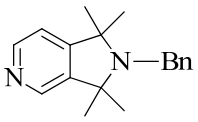

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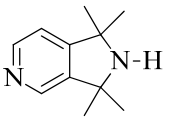

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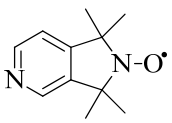

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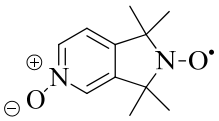

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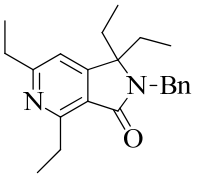

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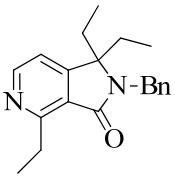

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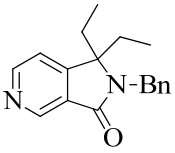

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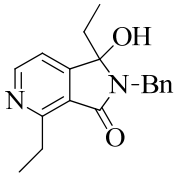

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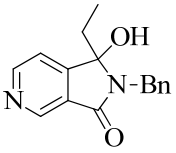

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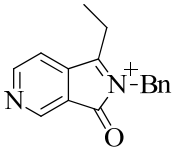

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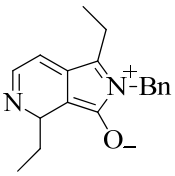

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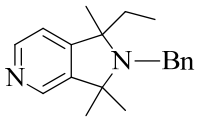

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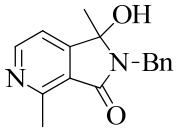

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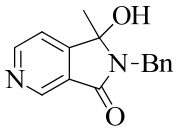

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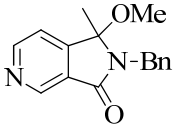

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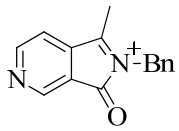

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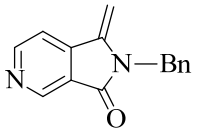

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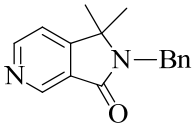

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